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The Role of the Thyroid in the Development of Platyfish[1]

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(Plates I-IX; Text-figures 1-4)

THE role of the thyroid in the physiology of fishes has been a subect of controversy for years (Lynn & Wachowski, 1951; Hoar, 1957; Pickford, 1957). Although a considerable body of evidence has accumulated in support of the importance of the thyroid in the growth and maturation of teleosts, there also have been many conflicting reports. Until recently, work in this field was hampered by the impossibility of thyroidal extirpation in most teleosts, owing to the diffuse nature of the tissue and its intimate relation to major blood vessels. Observations were limited to the study of effects of antithyroid drugs("chemical thyroidectomy") and of thyroid preparations that were administered to fish with autogenous thyroids (Table 12). The results of such experiments were always subject to criticism, because of the possibility of toxic effects exerted independently of the effect on thyroid hormone production, and because non-physiological hormonal excesses may have led to abnormalities.

Recently, radioactive iodine (I^{131}) has been employed as a thyroidectomizing agent in teleosts, with varying effectiveness (Table 11). However, the effects of replacement therapy in athyroid fish have not been reported. With proper controls, the use of I^{131} may permit analysis of thyroidal function in fish in a manner more nearly comparable to that made possible in higher animals by thyroidectomy.

This paper presents observations made, through the use of radioiodine treatment, on thyroidal function in the growth and sexual development of platyfish, *Xiphophorus maculatus*. The work presented here is an outgrowth of a series of experiments in which radiothyroidectomy of very young platyfish was attempted in an effort to obtain evidence relating to the origin of heterotopic thyroid tissue in this species (Baker, 1958a). Many of the radioiodine-treated fish and their controls described here were included in data reported in that publication, but only a few incomplete observations on their anatomy and pathology were given at that time.

Materials and Methods

1. *Radioiodine Treatment of Young Platyfish.* —The origins of the strains of platyfish used in these experiments have been described in Baker *et al.* (1955). Most of the experiments were made on fish of the *BH* strain, which was very susceptible to pharyngeal goiter and to thyroid tumors in the kidneys and other organs (Baker, 1958a).

a. *General Procedures*: (Table 1) Groups of platyfish, ranging in age from 17 to 70 days, were treated with radioiodine (I^{131}) by immersion in 200 ml. of aquarium water containing 4.5-5.02 mc of carrier-free I^{131}. The size of the treated groups ranged from 10 to 24 fish. Their standard length (tip of nose to end of caudal peduncle) was no more than 6 to 8 mm. and thus no deleterious overcrowding existed on a short-term basis. Young fish to be treated were carefully selected by eye, so that in treated and control groups the sizes of the fish were as evenly matched as possible. Exact measurements of length or weight were not made. The exposure time was varied between 24 and 72 hours, but in all but two of the 13 experiments, the exposure time was 48 hours or more.

Experiment 1 was a preliminary test of the efficiency of the treatment. These fish were of the 20th inbred generation of the *30* strain, which was highly resistant to goiters of all types.

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TABLE 1. EXPERIMENTS IN WHICH RADIOTHYROIDECTOMY OF YOUNG PLATYFISH WAS ATTEMPTED BY IMMERSION OF FISH IN WATER CONTAINING LARGE AMOUNTS OF RADIOIODINE

Experiment Number[1]	I^{131} conc. —mc./200 ml.	Hours Exposed	Age (days) at Exposure	Initial Fish Exposed	Exposed Fish Harvested		Initial Controls	Controls Harvested[2]	
					♀	♂		♀	♂
1.	5.5	48	60	12	0	8	.	.	.
2.	4.5	24	50	17	7	1	.	.	.
3.	5.0	48	63	21	4	3	.	.	.
4.	4.6	48	36	12	3	5	13	1	1
5.	5.0	48	37	12	7	5	11	3	8
6.	5.0	60	17	14	5	3	12	5	7
7.	5.01	72	66	12	2	2	11	1	1
8.	5.0	25.5	52	10	2	3	9	4	2
9.	4.82	60	58	13	0	0	8	0	0
I.	5.02	55.75	42	12	0	5	5	1	4
II.	4.99	52	56	12	0	6	6	2	3
III.	4.99	52	35	13	7	6	6	3	3
IV.	5.02	50	42	24	8	8	8	3	3
			Total:	184	100		89	55	

[1]Experiment 1 was performed on strain *30* fish, experiments 2 and 3 on strain *Fu* fish, and the remaining experiments on strain *BH* fish. No controls were kept in experiments 1 to 3.

[2]Discrepancies between initial controls and harvested controls in experiments 4 to 9 do not necessarily show final death rates, as controls were harvested in small groups at varying ages, for comparison with I^{131}-treated fish, and unused controls sometimes were returned to the breeding stock. See Table 4 for correct death rates among control groups.

Four of the fish in this experiment were sacrificed immediately post-exposure to determine radioiodine uptake per fish; the remainder were sacrificed at intervals of 6, 17 and 24 days post-exposure to determine the condition of the thyroid tissue. In all subsequent experiments, fish were not killed for examination until at least 40 days post-exposure.

Radioiodine uptake per fish was determined by counting aliquots of a 1N NaOH hydrolysate of the head, and aliquots of the radioactive water; per cent. uptake was obtained by the ratio of total counts in each. Total uptake was then obtained by multiplying per cent. uptake by the total original activity known to be in the water.

No specific controls were maintained in experiments 1 to 3; comparisons made were between treated fish and age-matched members of the same strain. In all other experiments, a closely similar number of broodmate controls was maintained. The controls were subjected to a "sham exposure," i.e., they were confined without feeding in a container in 200 ml. of water for the same period of time as the treated fish. The trip to the treatment site (Department of Zoology, Columbia University) was also taken by the controls, so that any effects of various shocks, such as cold, concussion, sudden darkness or light, would be the same.

In experiments 1 to 9, the treated fish, after exposure, were washed in radioiodine-free aquarium water and transferred to 4-gallon stock tanks, containing gravel, growing plants and snails, and were fed and maintained in the manner used throughout the Genetics Laboratory (Gordon, 1950). Control groups were maintained in adjacent tanks under similar conditions.

b. *Replacement Therapy:* In experiments I to IV, the radioiodine-treated fish were subsequently divided into two or three groups. One of these received no further treatment, while the others received potassium iodide or were fed desiccated thyroid. Non-treated broodmates were kept in equal initial number to each subdivision of the radioiodine-treated group. Further treatments of the I^{131}-exposed fish were begun 5-9 days after removal from the radioisotope. Fish that died during this interval are not included in figures comparing the variously treated groups, but only in total mortality data for radioiodine treatment.

In experiment I, the three groups of fish (control, I^{131}-treated, and I^{131}-treated subsequently given potassium iodide) were kept in stock tanks, and one group was given extra KI by the addition of 10 ml. of a 4 mg./ml. stock solution to its 4-gallon tank every two weeks. Therefore, until the tank water was changed, the concentration of remaining KI was unknown. In experiments II to IV, each subgroup was maintained in a specified volume of water (4-7 fish in two liters, or 8 fish in three liters) in all-glass aquaria, without gravel, plants or snails. These tanks were cleaned and the water changed weekly, and a known concentration of KI, where used, was employed (1 mg./l. final volume). Thyroid was administered by feeding ½ tablet of desiccated thyroid[3] weekly at the start of the experiments; other food was withheld on that day. This was increased to a whole tablet weekly as the fish grew (1 to 3 months after the start of thyroid-feeding, depending on size and number of fish in the group). On all other days, the fish were fed, together with the rest of the fish in the Laboratory, on dried shrimp or liver-pablum (Gordon, 1950). General observations on growth, body shape, coloration, secondary sexual development and "health and temperament" were noted during the weekly aquarium cleaning, when each group was transferred to a small observation vessel while its tank was washed and refilled. Whenever cleaning was done, the water was replaced from the Laboratory breeding tanks in such manner that each subgroup in an experiment received the same mixture of various tank waters.

Of 100 radioiodine-treated fish examined in these experiments, 87 were radioautographed, using tracer doses of I^{131} (Baker, 1958a), and all fish were serially sectioned for complete examination of internal organs.

2. *Radioiodine Treatment of Adult Platyfish.* —(Table 2). In these experiments, radioiodine was given by intraperitoneal injection to fully mature fish of both sexes. Since male playfish are smaller than females, and thus more difficult to inject, fewer males were used. Four groups, totalling 46 fish, were injected; each fish received 48.2 to 100 μc. of I^{131} in 0.012 to 0.05 ml. of distilled water. The first group was made up of 5 wild-type females of the *Fu* strain, the members of which were highly susceptible to thyroid tumors. One fish of this group gave birth to a few young 19 days after the injection; two of these young were radioautographed with the mother 22 days after birth and are described with the group. Groups 2 and 3 included females and males of the *163* strain, which was at least moderately susceptible to thyroid tumors. Group 4 consisted of 12 females of the *30* strain; these fish all died within 5 months post-injection and are included only in the mortality figures.

After injection, these fish and their broodmate controls were maintained under ordinary stock conditions. Twelve of the 18 radioiodine-treated fish that were harvested were also radioautographed, and all were serially sectioned for complete examination of internal organs.

3. *Radiophosphorus Treatment of Young Platyfish.*—As radiation controls for the I^{131}-treated young fish, 16 platyfish of the *BH* strain were placed in 200 ml. volumes of aquarium water containing 1.7 mc. of P^{32} for 48.5 to 50.75

[3]Burroughs-Wellcome & Co., "Tabloid" thyroid, U.S.P. Each tablet contained 0.065 gm. desiccated thyroid. These tablets were tested for metamorphic activity on tadpoles (three years after the fish experiments were completed). In three tests, ¼-2 tablets, fed to 4 or 5 three-centimeter *Rana pipiens* tadpoles, produced tail resorptive processes, hind and fore limb and foot development, modification of mouth parts, lung breathing, pigmentary differentiation, raised eyes and lack of further growth. Controls were unchanged within the period (6-11 days) of treatment.

TABLE 2. EXPERIMENTS IN WHICH RADIOTHYROIDECTOMY OF ADULT PLATYFISH WAS ATTEMPTED BY INTRAPERITONEAL INJECTION OF RADIOACTIVE IODINE (Starred (*) fish were radioautographed)

Group	Pedigree	Number of Injected Fish		μc. I^{131} Injected	Age (months) when Injected	Post-injection Period (months)	Number of Controls	
		♀	♂				♀	♂
1.	507 *Fu*							
	Fish started	5	0	Various	8.4		0	0
	Fish harvested	1*	0	80		1.4	0	0
		2*	0	50		6.5	0	0
2.	*163*^{8}							
	Fish started	11	.	96.4	8.7-9.5		10	.
		.	6	48.2	9.0-9.8		.	No record
	Fish harvested	1*	.			1.3	0	.
		2*	1*			3.5	2*	1*
		1	.			19.5	1	.
3.	*163*^{8}							
	Fish started	12	0	50	8.8-11.5		12	0
	Fish harvested	5*	0			8.5	0	0
		5	0			18.75	3	0

hours. These were groups of 4-7 fish, aged 30 to 54 days. The amount of P^{32} used was calculated to give the same roentgen dose of whole-body irradiation within the 200 ml. volume in 48 hours (720 r.) as would 5.0 mc. of I^{131} (Glasser *et al.*, 1952).[4] Fourteen untreated broodmate controls were kept. These fish were maintained in stock tanks after treatment and, when 4.7 to 7 months old, were fixed in formalin and serially sectioned for examination.

4. *Analysis of Pituitary Development.*—A ratio of pituitary to body size was obtained in several experiments, using counts of consecutive 10μ sections of the pituitary as a measure of hypophyseal size, and standard length of the fish, taken from the specimen itself or its photograph, as the measure of body size. The average ratios obtained by this method were often inconsistent between experiments, when variously treated groups were compared. A better measure of hypophyseal development was obtained by making a series of outline drawings of each section of the pituitary by means of uniformly magnified projections. The tracings from each fish were then cut out and weighed on an analytical balance. These weights were then averaged within each treatment group and divided by the average standard length within the same group. The ratios obtained by this method proved to be satisfactorily consistent between experiments with reference to the relations between the variously treated groups. Volume or weight measurements were not taken on any of the fish before sectioning, so that only length was available as a measure of body size. Objection to a ratio of volume: length, as involving two different types of measurements, may be countered, in these experiments, with the following observations: Had the proportions of all of the fish been identical, the use of this ratio would have been justified without doubt, but if not, it might have led to misinterpretation. However, the differences in proportion observed among the treated groups of fish usually would have *enhanced* the differences found by using the pituitary-volume-to-body-length ratio. For example, radioiodine-treated fish tended to be dumpy and pot-bellied in shape, which would have increased their weight or volume in proportion to their length, but if such an increased volume had been used to calculate the ratio, the difference between these fish and the controls would have become *greater* than indicated at present. This would also hold true for KI-treated, radioiodine-exposed fish, for the same reason. On the other hand, thyroid-fed fish, if slenderer than their controls, would have had their ratios shifted slightly towards those of the controls. The interpretation of the results obtained, in any case, could not have been affected seriously.

5. *Histological Methods.*—All fish were serially sectioned at 10μ and stained with haematoxylin-eosin or occasionally with Masson's trichrome. Fixation was routinely in Bouin's fluid with formic acid as a decalcifier, with the exception of the P^{32}-treated fish which were fixed in 10% formalin. Radioautography was carried out as described in Baker, 1958a.

6. *Gonopodial Development.*—In experiments I to IV, the anal fins of all fish were removed, after fixation of the whole fish in Bouin's fluid, dehydrated in alcohols and xylol, and whole-mounted on slides in Permount, unstained, for examination. This was also done with the anal fins of some animals from experiments 2-8 and with those of all P^{32}-treated fish.

Results

1. *The Efficacy of Radiothyroidectomy in Platyfish.*

a. *Criteria for Thyroidectomy:* Thyroidectomy was judged to be complete when I^{131} radioautographs of serial sections through the thyroid and kidney regions of the fish were blank for thyroid tissue. Radioautographic spotting usually was found also over the auditory region and portions of the pharynx and intestines. In the latter two locations, the spots were produced by I^{131} that was bound to food particles in the lumen of the alimentary tract or adhering to its lining (Pl. II, Fig. 1). These non-thyroidal sources of spotting were readily differentiated by their location from spots produced by thyroid tissue, even when crude contact radioautography was employed. Location of spots within a section was often aided by the occurrence of a "shadow picture" of the whole section, sometimes including definition of organ areas contrasting with open areas within the section.

In addition to the radioautography, all serial sections were searched histologically in a methodical fashion for thyroid tissue in the pharyngeal and renal areas. In some cases, when radioautography was not feasible, as in case of preservation after death, histological search was accepted as sole criterion for thyroidectomy. This was used in relatively few cases, however, and was related to the total morphological picture of the individual before any acceptance of

[4]I wish to thank Dr. Edith Quimby, Department of Radiology, Columbia University College of Physicians and Surgeons, for performing the initial calculation of the P^{32} activity required and for references to the relevant published material.

total thyroidectomy was made. In many instances, especially in younger fish, radioautographic speckling was noted, but no thyroid tissue could be found histologically. The probable presence of undestroyed thyroid tissue in these cases was revealed by later study of fish from the same group; in these older fish, visible thyroid tissue was often found. Thus, histological criteria for total thyroidectomy were not found to be completely reliable on a short-term basis.

b. *Radioiodine Treatment by Immersion:* On the whole, this method was very successful. In 92 fish examined 40 or more days after treatment, the frequency of complete radiothyroidectomy, by all criteria, was 68.5%. With the omission of two less successful experiments (Nos. 2 and 5), however, this incidence was 86% (62 of 72 fish harvested).

The results appeared to be affected both by exposure time and by the strain to which the fish belonged, when dose was nearly constant. For example, no fish were completely thyroidectomized in experiment 2, when strain *Fu* fish were exposed for 24 hours to 4.5 mc. I^{131}, but all fish were thyroidectomized in experiment 8, when strain *BH* fish were exposed for 25.5 hours to 5.0 mc. I^{131}. On the other hand, in experiment 3, most strain *Fu* fish *were* thyroidectomized by 48 hours exposure to 5.0 mc. I^{131}. The *BH* strain of platyfish was particularly highly sensitive to low iodine concentration in its environment, as judged by the frequency of thyroid tumors and heterotopic thyroid tissue (Baker, 1958a, b), and the fish probably concentrated iodide in the thyroid tissue, when supplied in excess, to a greater degree than platyfish of other strains.

The poor results of experiment 5 perhaps were related to an addition of $NaHCO_3$ to the radioiodine solution. This was the only apparent difference between this and later experiments using fish of the *BH* strain of similar age as well as closely similar amounts of radioiodine and exposure times. Although none of these fish was completely thyroidectomized, as judged by spotting on radioautographs, the amount of thyroid tissue was severely reduced, and none could be found histologically in many cases.

Among the group of 29 fish accepted as *incompletely* radiothyroidectomized, thyroid follicles were unquestionably seen in 18 animals (Table 3). In the remaining 11 fish, radioautographic spotting, together with visualization of a few doubtfully identified thyroid follicles in some fish, comprised the evidence for incomplete thyroidectomy. These doubtful cases were largely confined to animals that were examined within three months after I^{131} treatment and were no more than 4.5 months old. Among fish

TABLE 3. COMPARISON OF CRITERIA FOR COMPLETENESS OF THYROIDECTOMY IN PLATYFISH TREATED WITH RADIOIODINE BY IMMERSION[1]

Experiment Number[2]	Total No. Fish Harvested	Days Post-treatment when Examined	Number of Incomplete Thyroidectomies, Judged from: Radioautograph Spots, No Follicles Seen	Number of Incomplete Thyroidectomies, Judged from: Follicles Seen Histologically
2	8	41	0	3
		150	0	2
		176	0	1
		539	0	2
3	7	47	1	1
		171	0	1
5	12	79	2	2
		95	5	0
		168	1	1
6	8	60	1	1
		277, 289	0	1
I	5	159	1	1
IV	16	140	0	2[3]
Total of incomplete thyroidectomies:			11	18

Fraction of incomplete thyroidectomies: 29 out of 92 I^{131}-treated fish harvested in all experiments, except No. 1.

[1]*All* fish radioautographed 40 or more days after treatment. The number of *incomplete* thyroidectomies that could be shown by radioautographic criteria exceeded by a large increment the number that could be demonstrated by histological examination alone.

[2]In experiments 4, 7, 8, II and III all fish were totally thyroidectomized. Therefore these experiments are not listed here.

[3]One of these had only a single thyroid follicle, but in the kidney.

more than 6 months old there rarely was any question about the presence of undestroyed thyroid tissue. Not only did the radioautographic spotting per follicle become unusually intense, but the amount of thyroid tissue often became considerable (Pl. II, Figs. 3, 4). Whenever visualized and in whatever quantity, thyroid tissue in the non-thyroidectomized fish was hypertrophied and the colloid was granular and small in amount. The two oldest fish were 19.6 months of age, and in them the thyroid tissue had assumed almost goitrous proportions, although each follicle still remained distinct and slightly separated from others and did not form a continuous mass of follicular and afollicular tissue as seen in most goiters among platyfish (Pl. II, Fig. 4).

In the pilot experiment (No. 1), 12 young fish of a strain insensitive to low concentrations of environmental iodide were used. Four of these fish were killed immediately after exposure and the radioiodine uptake per fish was determined. This proved to be 20-50 μc. Two fish were examined on the 6th day after treatment, two on the 17th day and four on the 24th day. On the 6th day, the number and distribution of thyroid follicles appeared to be normal. The morphology of the follicles was normal, but the colloid often stained blue with haematoxylin-eosin. Normally, thyroid colloid stained bright red with this stain combination. The blood vessels in the thyroid area were normal, and no other anatomical changes were noted. By the 17th day, the radioautographs were "spotty" and the number of thyroid follicles was severely reduced. The cells of these follicles were heightened, often puffy, with very indefinite boundaries and pale cytoplasm. The colloid was pale pink and coarsely granular. No follicles had normally dense colloid or colloid stained blue. The aorta and walls of the gill chamber in the thyroid area were somewhat thickened. No other pathological effects were noted. On the 24th day post-treatment, one fish was completely lacking thyroid by all criteria, one had a single spot on the autograph but no visible thyroid follicles, and two retained a few identifiable follicles. The latter were often very indistinct and had pale, enlarged cells and pale-pink granular colloid. In one fish, the thyroid may have begun to recover as there were two or more follicles with dense vacuolated colloid and flat cells, as well as several pathological follicles. In all of these fish, the aorta was very shrunken, its walls were thickened, and it was surrounded by a thick layer of gelatinous-appearing connective tissue which attached it to the walls of the anterior pericardial space (Pl. II, Fig. 6). One fish had some enlarged tubules containing concretions in the kidneys (the kidneys of 2 of the 4 fish were not sectioned). The positive results obtained in this trial, with fish of a goiter-resistant strain, provided a basis for repetition of the method, using the goiter-prone strains, which might be expected to take up radioiodine more avidly, or release it more slowly, and therefore be more effectively treated.

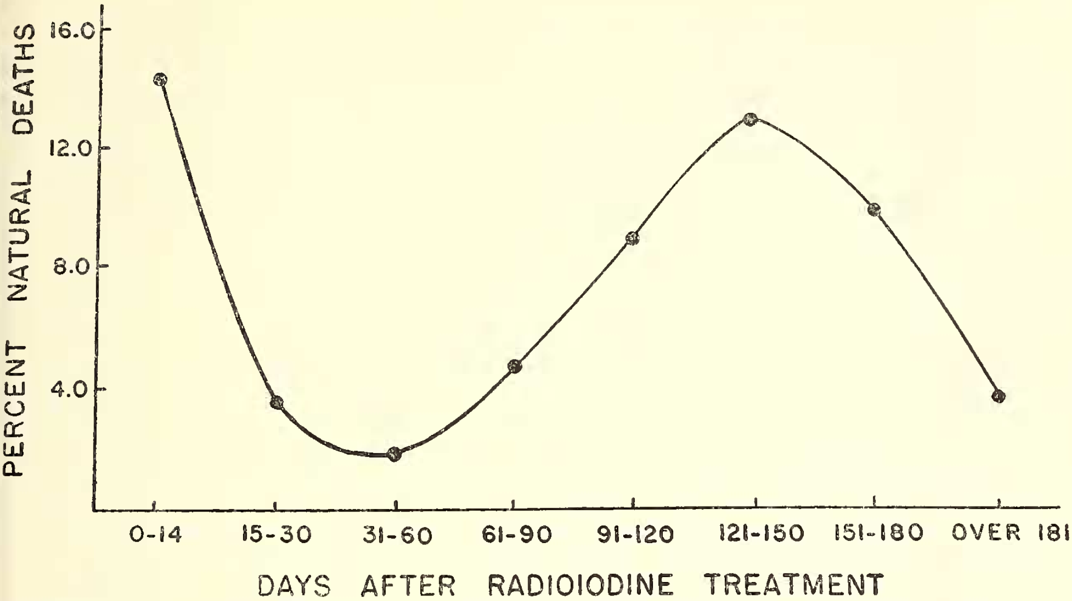
c. *Radioiodine Treatment by Injection:* (Table 2). This method was only moderately successful in producing thyroidectomized fish, although it produced severely hypothyroid animals in all cases. The chief difficulties encountered were the necessity for keeping the injected volume very small, especially for male fish, which are smaller than females, and the tendency for post-injection leakage to occur. Since relatively few fish were treated by this means, and because of the lesser importance of these experiments to the analysis of the chief problem in hand at the time, these difficulties were never fully dealt with. Nevertheless it is likely that successful administration of the dosages intended for the fish would not have brought about much different results since various amounts of I^{131} in various volumes were injected, with fairly similar effects.

Here again, strain differences were noted. The three female *Fu* fish of Group 1 were quite successfully thyroidectomized by injections of 50-80 μc. of I^{131}, whereas in Group 2, none of the *163* female fish was completely thyroidectomized by injections of 96.4 μc. of I^{131}. Fish of the *163* strain produced renal thyroid tumors, but with a much lower frequency than did the *Fu* strain. From this it might be assumed that the thyroids of *163* fish had a lower avidity for, or retention of, iodine than those of *Fu* fish.

Among the 18 fish harvested (Table 2), total *histological* thyroidectomy was obtained in 9 animals, but two of these had died in I^{131} tracer solution before fixation, so that the preservation was poor. Radioautographic total thyroidectomy was obtained in only one instance. Two fish were examined 38-41 days post-injection. From Group 1 (strain *Fu*), one fish was lacking thyroid histologically, although it retained a faint radioautographic speckling in the thyroid area. From Group 2 (strain *163*), one fish showed thyroid tissue histologically, although extremely reduced. The thyroid cells were high and the colloid was pale and granular. All other fish were examined 3.5 or more months after the injections.

2. *The Effects of Radioiodine Treatment on Young Platyfish.*

a. *Mortality:* The death rate among treated fish in these experiments was very high. Of 172



TEXT-FIG. 1. The distribution in time of deaths among radioiodine-treated platyfish. The percentages are based on corrected totals for remaining fish, after subtraction of those killed for examination in each time period. Therefore, the figures do not add up to 100%. The analysis is based on an initial 113 fish, from those experiments in which all deaths were recorded with respect to the time periods (experiments 2, 5, 8, 9, and I-IV). Of these fish, 59 were killed after the 30th day post-treatment, and 54 died "naturally."

radioiodine-treated fish, in all experiments except No. 1, 89 (52%) died during maintenance periods that ranged from 128 to 290 days. These deaths were largely concentrated in the periods between 0 to 2 weeks post-exposure, and between 3 to 5 months post-exposure (Text-fig. 1). A more accurate picture of survivorship, uncomplicated by early sacrifices and lack of control data, is obtained by the analysis of experiments 7-9 and I-IV only, in which all fish were maintained untouched until the conclusion of the experiment. Survivorship to the 109th and 130th day post-exposure for these experiments is shown in Table 4. (The 109-day survivorship is presented for comparison with mortality data on P^{32}-treated fish, most of which were kept for only 109 days post-treatment. See below, part 4). Comparison of the 109-day with the 130-day survivorship further illustrates the heavy loss of treated fish between three and five months after treatment. The death rate among the untreated controls in these experiments was almost negligible.

b. *Growth:* Among the most prominent effects of radioiodine treatment was a striking reduction in the growth of the treated fish in comparison with untreated broodmates. Examples of differences in average standard length between treated and control fish, at various ages, are given in Table 5. These differences may be also seen in Pl. I, Fig. 2. There was little or no difference between the 2.5-month-old treated and control fish of experiment 6, but in older fish differences of more than 10% were found in 5 out of 6 groups. It must be borne in mind that mortality among the treated fish was invariably highest among the smaller fish, so that the final survivors were a selected group of the largest size. Among the controls, deaths were also most frequent among the smallest fish, but very few of any size died. No measurements of growth rate were made, only the size attained at fixation being recorded.

Radioiodine-treated fish were frequently observed to develop a hunched, "cretinous" body shape, with a pot belly. This may be seen in Pl. I, Figs. 1 and 3, in both living and fixed specimens. The pot-bellied appearance was found to be related to the condition of the liver and the abdominal tissues (see below). Treated fish also were noticeably darker in color than the controls in most cases, but this difference was not further investigated.

c. *General Pathology:* The ventral aorta and the bases of the afferent branchial arteries, the bulbus arteriosus and other arteries passing through the thyroid area were usually shrunken in radioiodine-treated fish. The walls of these

TABLE 4. SURVIVAL RATE IN PLATYFISH TREATED BY IMMERSION IN WATER CONTAINING LARGE AMOUNTS OF RADIOIODINE[1]

Experiment	Fish Treated	Survivors	Controls Kept	Control Survivors
A. Survivorship to the 109th day after treatment:				
7	12	9	(11)	Incomplete record
8	10	10	9	9
9	13	8	8	8
I	8	3	5	5
II	5	3	6	5
III	7	7	6	6
IV	8	6	8	7
Total:	63	46	42	40
Percent. Survival:		73[2]		95
B. Survivorship to the 130th day after treatment:				
7	12	4	(11)	Incomplete record
8	10	10	9	9
9	13	2	8	8
I	8	3	5	5
II	5	2	6	5
III	7	5	6	6
IV	8	6	8	6
Total:	63	32	42	39
Percent. Survival:		50		93

[1]These figures do not include fish that were additionally treated with potassium iodide or fed thyroid material.

[2]Percent. survival would be 70 if two fish in experiment 7, whose deaths were inexactly recorded, had died before the 109th day.

vessels, particularly the aorta, were thickened, and connective tissue "adhesions" between the aorta and the pericardial lining frequently were present and developed to a considerable extent (Pl. II, Fig. 5). In some fish, this material formed a thick, structureless, gelatinous-appearing mass around the aorta (Pl. II, Fig. 6). The heart was never visibly affected. The area of the thyroid often was filled with fibrous stroma, and in some fish the caudal portion of the area, above the aorta, disappeared through the collapse of the walls of the gill chamber into the vacant space.

Most I^{131}-treated fish suffered a loss of lymphoid tissue in the kidneys to some extent. These are the chief blood-forming organs in teleosts, with the spleen second in importance. In most of the fish, this loss was not severe, but definitely was noticeable upon histological examination. In general the spleen seemed to be normal. No lymphoid cell counts or blood counts were made on these fish. In a few treated fish, almost complete loss of lymphoid elements was found. This was first apparent from the empty appearance of the kidneys (Pl. III, Fig. 2), and was accompanied by a severely shrunken spleen (Pl. III, Fig. 7) and an absence of thymus tissue.

The thymus was found to persist as a pair of large lymphoid organs in all fish that were totally radiothyroidectomized, regardless of sex, with the exception of those specimens just described. The thymus glands of normal platyfish are very large and solid structures in late embryonic and immature post-natal stages. As the fish mature, these glands often develop a few large internal cysts, sometimes filled with an eosinophilic colloidal material. In some instances, these cysts closely resembled thyroid follicles, but they were not found to produce I^{131} radioautographs. They evidently correspond to the cysts and follicular or alveolar structures described in the thymus of *Necturus* by James (1939). As the male gonad becomes fully mature and full of sperm, the thymus glands shrink and may disappear entirely. In female fish, however, large thymus glands were found at all ages up to 20 months, but they more often were cystic in females 9 or more months of age. Pregnancy had no noticeable effect on the thymus. In fish that were not totally radiothyroidectomized, the thymus glands changed in the normal, characteristic way, as sexual maturation took place.

Although the glomeruli and the bulk of the kidney tubules were not visibly affected, the latter structures in the kidneys of I^{131}-treated fish

TABLE 5. DIFFERENCE IN GROWTH BETWEEN RADIOIODINE-TREATED FISH AND THEIR BROODMATE CONTROLS[1]

Experiment	Age (months)	Number of Treated Fish	Mean Length: Treated Fish	Range in Length: Treated Fish	Number of Controls	Mean Length: Controls	Range in Length: Controls	Difference: Control Mean Minus Treated Mean	Difference: as % of Control Mean
5	4.4	5	17.4	16.5-17.5	5	21.9	20 -24.0	4.5	20.5
6	2.5	4	10.5	10.0-11.0	3	9.9	9 -10.8	−0.6	−6.1
8	9.6	5	15.5	15.0-16.0	6	24.0	20 -26.0	8.5	35.4
I	6.8	2	28.8	27.5-30.0	5	32.1	29 -35.5	3.3	10.3
II	6.3	2	20.8	19.5-22.0	5	21.4	15 -26.0	0.6	2.8
III	6.3	4	17.8	15.0-22.0	6	23.2	20.5-27.0	5.4	23.3
IV	6.1	4	14.5	11.0-16.5	6	16.9	10.5-20.5	2.4	14.2

[1]Standard length (tip of nose to end of caudal peduncle) was measured in millimeters. In experiments 5-8, true measurements were taken just after fixation of the fish; in experiments I-IV, measurements were made on group photographs taken for each experiment after the fish were fixed (see Pl. I, Figs. 2, 3) and do not represent the true lengths of the fish. The measurements made on photographs are not comparable between experiments, because the magnifications are not identical.

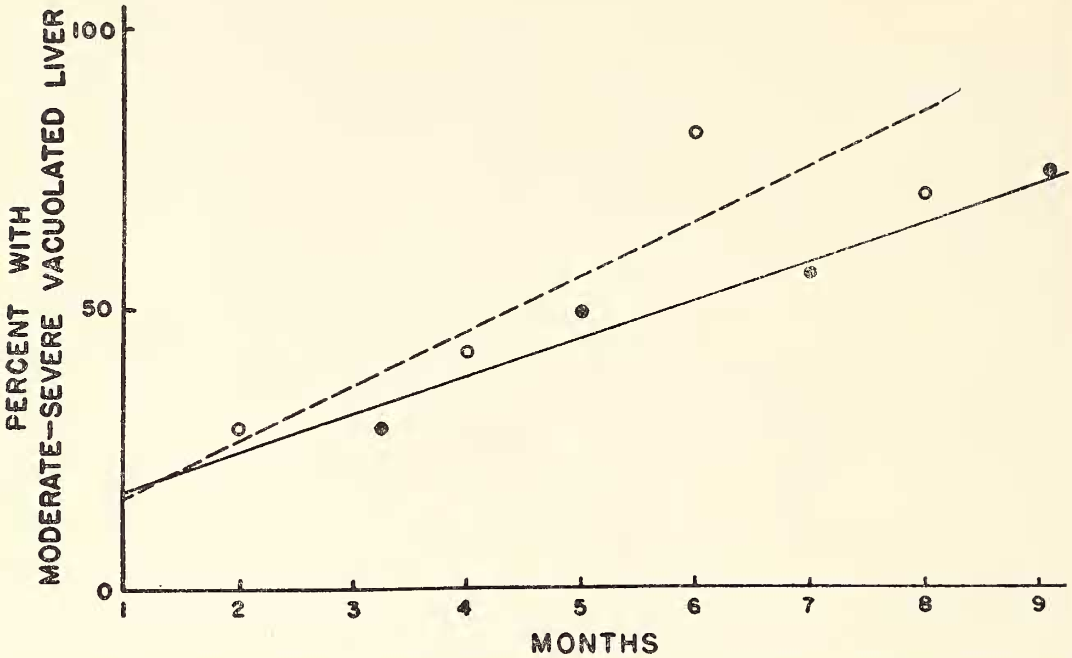
were in part pathological. Many tubules were swollen and their lumens contained unstained masses of material best described as "concretions" (Pl. III, Figs. 3, 4). Tubules also were seen to be degenerating, and sometimes small wormlike structures which were very darkly basophilic were seen (Pl. III, Fig. 6). These are believed to be regenerating tubules.

The liver of radioiodine-treated fish was usually the most noticeably and severely affected internal organ. The pot-bellied appearance of these fish resulted from an enlargement of the liver, which was often extreme, coupled with greatly increased abdominal "vacuolation." In these enlarged livers, "vacuolation" was found to pervade the entire organ to a remarkable extent (Pl. IV, Figs. 2, 4). The term "vacuolation" will be used throughout this paper to describe the condition of the liver and abdominal tissue in many radioiodine-treated fish. Although it is believed that this condition probably represented fatty deposits, the presence of fat could not be demonstrated because of the routine processing of all material in fat solvents, such as xylol. No frozen sections stained for fat were prepared, as the liver condition was not analyzed at the time when fresh experimental material was available.

Vacuolated livers were found in a large proportion of the treated fish; severity ranged from small vacuolated areas in the antero-central portion of the liver to complete change of the entire organ. Severity was found to be correlated with age (or to time elapsed after treatment) in a remarkably linear fashion (Text-fig. 2). Vacuolated livers were found in incompletely thyroidectomized fish with nearly as high a frequency as in totally thyroidectomized fish (Table 6), but never was found among the controls, even in the presence of thyroid hypertrophy.[5]

Included among the fish with vacuolated livers are several specimens whose livers contained large amounts of ceroid (Pl. IV, Fig. 3). The ceroid found in the I^{131}-treated platyfish occurred in globules in the liver cells themselves, and also in large masses of large, dark-nucleated cells, which were solidly filled with it. This observation concurs with the distribution in experimentally cirrhotic rats reported by Endicott & Lillie (1944), who described ceroid as occurring largely in large round phagocytes which formed broad sheets and trabeculae. In the I^{131}-treated

[5]Vacuolated livers were found in many platyfish that had thyroid tumors in their kidneys, although not in all specimens. Most of these fish were relatively old animals.



TEXT-FIG. 2. Increase with time in severity of liver vacuolation (see text) among radioiodine-treated platyfish. The fish were classified into four groups of increasing severity from their histological descriptions, and then subgrouped by age and by the period elapsed after treatment. When the two most severely affected groups were compared with the two least severely affected, a straight line relationship to age emerged, and a similar, though less regular, relationship to post-treatment time appeared. These curves show that long-continued hypothyroidism had a progressive deleterious effect on liver function. Pathological effects of radiation, on the other hand, show recovery in time, if the animal is able to survive. Solid circles and continuous line represent age, and open circles with dashed line represent time post-treatment, both on the same time scale.

fish, broad masses of ceroid-filled cells also were found near hepatic blood vessels and among the pancreatic tissue outside of the liver. In some fish, vacuolated areas and extensive ceroid deposits were found together in the liver.[6] In the case of the radioiodine-treated platyfish, it was supposed that the conditions of vacuolated liver and "ceroid liver" were related, and, if the assumption is made that vacuolation does represent fat, available information appears to support this view (see Discussion). In normal fish, ceroid also was seen in small aggregations of macrophages next to blood vessels, but never was seen in liver cells.

[6]A re-examination of the livers of 8 thiourea-treated platyfish and their 9 controls (originally described in Baker, 1958b) was made. Ceroid-filled macrophages were found massed in the livers of all treated fish and to an extreme degree in 7 of them. Ceroid deposits, besides those in macrophages, were found in the liver cells themselves. Prominent vacuolization, coupled with ceroid deposition, occurred in 4 fish, and in two of these cases there was severe cellular degeneration in the central parts of the liver. In the controls, ceroid was absent from liver cells and scant in macrophages, while vacuolated livers did not occur.

In fish with extensive vacuolation in the abdomen, the pancreatic tissue was severely compressed and may best be described as wispy (Pl. IV, Fig. 6). In most cases, however, the amount of pancreatic tissue appeared to be relatively normal.

d. *Gonadal Development:* No completely radiothyroidectomized fish ever attained sexual maturity, even at an advanced age (there were three possible exceptions, which will be described below, but these fish were not conclusively athyroid). Gonadal development in treated fish and controls is shown in Tables 7 and 8. From the youngest fish sampled, including incompletely thyroidectomized ones, it was apparent that gonadal development was retarded in all I^{131}-treated fish (Pl. V, Figs. 1, 2). Among completely athyroid animals, this retardation frequently was extreme. As shown in Table 8, many males had developed no primary spermatogonial cysts (Wolf, 1931) by the age of 6 months or more (Pl. V, Fig. 4), when controls were producing young, and many treated females had developed only a few early basophilic oocytes (Pl. V, Fig. 6). Although some thyroidecto-

TABLE 6. INCIDENCE OF VACUOLATED LIVERS (SEE TEXT) IN RADIOIODINE-TREATED PLATYFISH AND THEIR BROODMATE CONTROLS

	Number of Fish	Number with Vacuolated Liver	Percent. with Vacuolated Liver
Fish 5 or less months old:			
Complete thyroidectomy	7	3	43
Incomplete thyroidectomy	8	5	63
Doubtful	9	7	78
Total I^{131}-treated	24	15	63
Controls	11	0	0
Fish 5.1 to 7 months old:			
Complete thyroidectomy	24	17[1]	71
Incomplete thyroidectomy	5	3	60
Total I^{131}-treated	29	20	69
I^{131} + KI treatment	9	6	67
I^{131} + thyroid feeding	15	0	0
Controls	30	0	0
Fish more than 7 months old:			
Complete thyroidectomy	9	8	89
Incomplete thyroidectomy	3	2	67
Total I^{131}-treated	12	10	83
Controls	13	0	0
	—	—	—
Grand total solely I^{131}-treated:	65	45	69
Grand total controls	54	0	0

[1]One additional fish with other abnormalities of the liver not included.

TABLE 7. GONADAL DEVELOPMENT IN RADIOIODINE-TREATED PLATYFISH AND THEIR BROODMATE CONTROLS

Experiment	Age (months)	Radioiodine-treated			Controls		
		Total	Mature	Immature	Total	Mature	Immature
A. Total Thyroidectomy							
3	6.8	2	0	2	.	.	.
	8.4	1	0	1	.	.	.
4	6.0	4	0	4	2	2	0
6	7.5	2	0	2	.	.	.
	10.2	1	0	1	7	7	0
7	6.5	4	0	4	2	2	0
8	9.7	5	0	5	6	5	1
I	6.8	2	0	2	5	5	0
II	6.3	2	0	2	5	3	2
III	6.3	6	0	6	6	4	2
IV	6.1	5	0	5	6	1	5
		—	—	—	—	—	—
Total:		34	0	34	39	29	10
Percent. Mature:			0%			74%	
B. Incomplete Thyroidectomy							
2	6.6	2	2	0	.	.	.
	7.5	1	1	0	.	.	.
3	7.8	1	1	0	.	.	.
5	5.7	1	1	0	.	.	.
	6.8	2	2	0	1	1	0
6	9.8	1	1	0	.	.	.
		—	—	—	—	—	—
Total:		8	8	0	1	1	0
Percent. Mature:			100%			100%	

TABLE 8. DEGREE OF SEXUAL DEVELOPMENT AMONG TOTALLY RADIOTHYROIDECTOMIZED PLATYFISH MORE THAN 5 MONTHS OF AGE[1]

Experiment	Females with: ≦ 5 oocytes	Females with: > 5 oocytes	Males with: Spermatogonial Cysts Absent	Males with: Spermatogonial Cysts Present
3	2	1	.	.
4	1	0	2	1
6	.	.	3	0
7	3	0	1	0
8	0	2	1	2
I	.	.	2	0
II	.	.	0	2
III	2	2	0	2
IV	0	2	3	0
Total:	8	7	12	7

[1]None of these fish was mature: females showed no signs of yolk deposition, while males possessed no germ cells in late stages of meiosis or in sperm formation. Some of the males in which spermatogonial nests or cysts were found exhibited a few cysts with cells in early spermatogonial division. Radioiodine-treated fish that were treated secondarily with potassium iodide or were fed thyroid have been excluded.

mized fish were maintained until 8 to 10 months of age, gonadal development proceeded no further than the enlargement of a few oocytes, without yolk deposition, in females (Pl. VI, Fig. 1), and the beginning of germinal cyst development, with some early meiotic cysts, in males (Pl. VI, Fig. 2). On the other hand, *all* fish with incompletely destroyed thyroid tissue were sexually mature by the age of 5 months, although no females were found to be gravid (note, however, that only in experiment 2 were treated females and males, that were maturing normally, living together).

Development of the gonopodium (the structurally specialized anal fin found in the males of this live-bearing species of fish, which is used for internal fertilization of the female) in thyroidectomized male fish was inhibited along with the testes, the anal fin remaining juvenile in form and undifferentiated. In one case, where an extremely infantile testis was present, there was elongation of the anal fin, but no segmental differentiation of the rays or of the specialized structures at the tip of the fin had occurred (Text-fig. 4; Pl. VII, Fig. 4). This type of anal fin development was described in gonadless hybrids between *Xiphophorus helleri* and *X. maculatus* by Rosen (1960).

Two of the exceptional I^{131}-treated fish mentioned above were males that developed large mature testes, with spermatophore production, in the apparent absence of thyroid tissue (Pl. VI, Figs. 3, 4). One, aged 6 months (experiment 5), unfortunately died and was not radioautographed, but the sections were remarkably good and were stained in a most striking way for thyroid delineation (Masson's trichrome). The other had been treated with potassium iodide (see below, experiment I) and was radioautographed, but KI is known to interfere with the uptake of further (radioactive) iodide (Baker, 1958b). Both of these fish had anal fins that were elongated but completely undifferentiated (Pl. VII, Fig. 4). Both also had among the most extremely vacuolated livers seen (Pl. VI, Fig. 4), with large aggregations of vacuoles compressing the pancreatic tissue in the abdomen, and with concretions in the kidneys. The KI-treated fish had developed the bright red color characteristic of most males of the *BH* strain (females and young were more yellowish or brownish).

The third possibly exceptional fish was a female, also from experiment 5, aged 7 months, with maturely yolked oocytes in the ovary. Although there were a few spots on the radioautographs, thyroid tissue was not positively identified histologically. This fish also had some vacuolization in the liver, extremely large abdominal aggregations of vacuoles, and concretions and degenerating tubules in the kidneys.

Anomalous development of the testes was found in 4 fish of the radioiodine-treated groups. Two of these were KI-treated fish of experiment I (see below), the others fish of experiments 5 and II, treated only with radioiodine. Three of these presented the same picture: an anterior testicular mass suspended solitarily from the dorsal peritoneum and unconnected by any discernible duct to the cloacal area or to a slightly posterior second testicular mass. The latter continued with a normal spermatic duct, opening close to the anus. In one case, both masses appeared to be bipartite. In the 4th fish (experiment 5), both testes appeared normal, but no spermatic duct could be found. None of these testes were near maturity. Similar anatomical peculiarities were not found among the controls.

e. *Pituitary Development:* No cytological studies were made on the pituitaries of I^{131}-treated fish because the fish were serially sectioned with the utmost rapidity in order to make radioautographs, and they therefore were cut relatively thickly (10μ). Staining also was routine; special methods were not used. The only cytological distinction that could be made was between a darkly basophilic cell type in the most anterior portion, a paler basophilic type in the mid-region, and the neural hypophysis. Eosinophils were not clearly recognizable because of the thickness of the sections and irregularities in staining.

A volumetric study of the hypophysis was attempted, however, and this produced rather clear-cut results (Table 9). It may be seen that the radioiodine-treated fish had significantly smaller pituitaries per unit of body length than did their controls. The pituitary:body size ratio remained constant at a low level as the I^{131}-treated fish grew older (in Table 9, experiments 5 and 8 may be compared), while the pituitary size of the controls increased in relation to their body length. It had been believed that the hypophysis of severely hypothyroid or completely athyroid fish might have been enlarged, owing to stimulation of thyrotrophs by the lack of thyroid hormone, but this was not the case. Instead, the size and appearance of the pituitaries suggested exhaustion, and the absence of any cellular increase in response to the environment.

The hypophyses of the control fish became larger per unit of body length as the fish grew older and at the same time that thyroid hypertrophy appeared in many of them. Both features presumably resulted from continued exposure to a low-iodine environment. The large relative size of the glandular portion of the hypophysis in the controls may be seen in Text-figure 3. This was particularly marked in female fish; which is in accordance with the observed higher incidence of goiter in females of the *BH* strain (Baker, 1958a) . The sex ratios among the treated and control fish were sufficiently alike to preclude any strong bias on a sex basis. Among the control fish, several had developed a marked overgrowth of the paler, intermediate basophiles, so that these overlapped the darker anterior basophiles ventrally, causing the appearance of a sharp line of demarcation in the anterior region and a distortion of the usually round cross-section of the hypophysis at that point (Text-fig. 3; Pl. VI, Figs. 6, 7). This appearance of the hypophysis was usually found in the presence of thyroid hypertrophy.

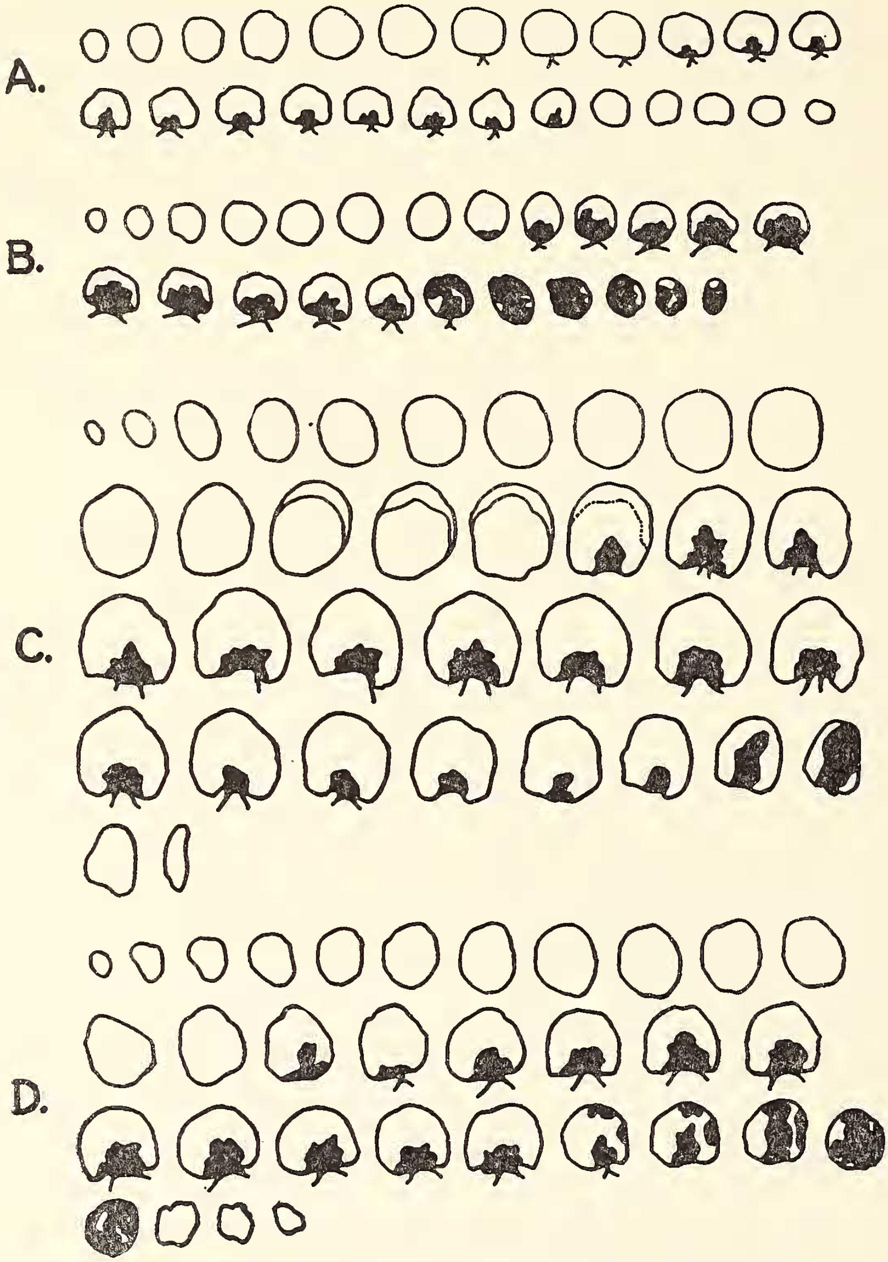
f. *Behavior and Fragility:* Although these observations are somewhat subjective and were not measured in a controlled manner, it is believed worth recording that the radioiodine-treated fish were very sluggish. They never were seen darting about the tank, and when caught for tank changing, were listless in avoiding the net. From general observation and two specific incidents, it was concluded also that they were easily killed by the shocks of handling and other disturbances. The first incident was an attempt to run two treated and two control fish in a Warburg apparatus. This also entailed transportation in a small container by 'bus over a considerable distance. The two treated fish died within an hour of being individually set up in Warburg vessels, although the rate of shaking used was very low. The two controls, on the other hand, remained alive and healthy for two days under identical conditions and were removed apparently none the worse for the experience. The second incident was the subjection of a treated fish to photography, together with a pair of normal broodmates (Pl. I, Fig. 1). This fish was manipulated for some time to obtain two

TABLE 9. PITUITARY VERSUS BODY SIZE IN RADIOIODINE-TREATED PLATYFISH, THEIR CONTROLS, AND IN RADIOIODINE-TREATED FISH FED THYROID[1]

Experiment	Age (months)	Solely I^{131}-treated	I^{131}, plus Thyroid-feeding	Control Fish
5	4.4	16.6 (3/2)	....	27.9 (0/5)
II	6.3	14.5 (0/2)	19.9 (0/4)	29.4 (2/2)[2]
III	6.3	13.1 (2/2)	11.7(3/3)	24.5 (3/3)
8	9.6	16.9 (2/3)	....	35.8 (2/4)

[1]Pituitary size was measured volumetrically and body size was measured as standard length. A ratio of the averages of the two parameters is tabulated here, for each group of fish. The number of males and females in each group is given in parentheses, as females/males.

[2]One fish of unknown sex not included. Its gonad may have been torn out when the anal fin was removed for mounting.



TEXT-FIG. 3. Projection drawings of serial sections of the hypophyses of representative strain BH platyfish from different experimental groups, all to the same magnification. The hypophyses were cut in cross-section, and are drawn with the ventral aspect uppermost. A comparison of over-all size and neural versus glandular development may be made. **A**, Radioiodine-treated immature female; **B**, Thyroid-fed radioiodine-treated mature female; **C**, Control (pregnant) female; **D**, Control mature male. It may be seen that the neural hypophysis (solid black areas) in the thyroid-fed fish appears to be nearly as large as that in the controls, but the glandular portion is much smaller. The glandular hypophysis in the normal females usually was much larger than in the normal males, although the male represented here had a slightly larger than average glandular portion; this may be related to the higher incidence of goiter in females of the strain. The hypophyses shown here were selected both for their representation of their respective groups, and for the most complete and undamaged series of sections.

photographic exposures. The animal was found to be dying the following morning and had to be fixed without autography to prevent its loss for examination.

3. *Replacement Therapy Experiments with Young Radioiodine-treated Platyfish.*

a. *Thyroid-feeding Experiments:* In these experiments, 18 radioiodine-treated fish were fed on desiccated thyroid once a week. The effects of this treatment were striking (Table 10). Mortality was reduced to a figure insignificantly different from that of the untreated controls, and the growth in body length equaled that of the controls, being significantly greater than that of the fish treated only with I^{131}. Body shape was normal, with a tendency towards a more streamlined appearance than shown by the untreated controls. This is enhanced in the photographs by the greater length of the caudal fin (Pl. I, Fig. 2) but the growth of the fins was not noted in the living fish and consequently the tails were not saved or measured. Examination of the photographs taken of each of these groups of fish suggests that the pectoral fins were also elongated, but these are often twisted from fixation and one cannot be sure. No sign of exophthalmia was found in the thyroid-fed fish.

These fish produced blank or nearly blank radioautographs, and the most meticulous histological search revealed no thyroid tissue. This also was true of the radioiodine-treated controls.

Most of these thyroid-fed fish showed a moderately to severely shrunken ventral aorta, with thickening of its walls and fibrous adhesions to the pericardium. In other respects they differed but slightly from the untreated controls. Very little lymphoid loss was seen in the kidneys or elsewhere, no degenerating tubules or concretions were found in the kidneys, and no vacuolization of the liver or abdominal tissue was present.

The sexual development in the thyroid-fed fish was most striking. Not only did fish of both sexes reach maturity by the end of the experiments, in equal or greater proportion to the untreated controls, but external male secondary sexual characters were often apparent considerably earlier than in the untreated controls (Table 10). Gonopodial structure was perfectly normal in the thyroid-fed males that matured before termination of the experiments (Text-fig. 4; Pl. VII, Fig. 2). At the close of the final experiment (IV), one of the thyroid-fed females was found to be gravid (in these small treatment groups, the sexes were not separated) (Pl. VIII, Figs. 3, 4).

TABLE 10. REPLACEMENT THERAPY OF RADIOIODINE-TREATED PLATYFISH. A SUMMARY OF THE RESULTS OF 4 EXPERIMENTS IN WHICH RADIOIODINE-TREATED FISH WERE SUBSEQUENTLY TREATED WITH POTASSIUM IODIDE OR WERE FED THYROID TABLETS[1]

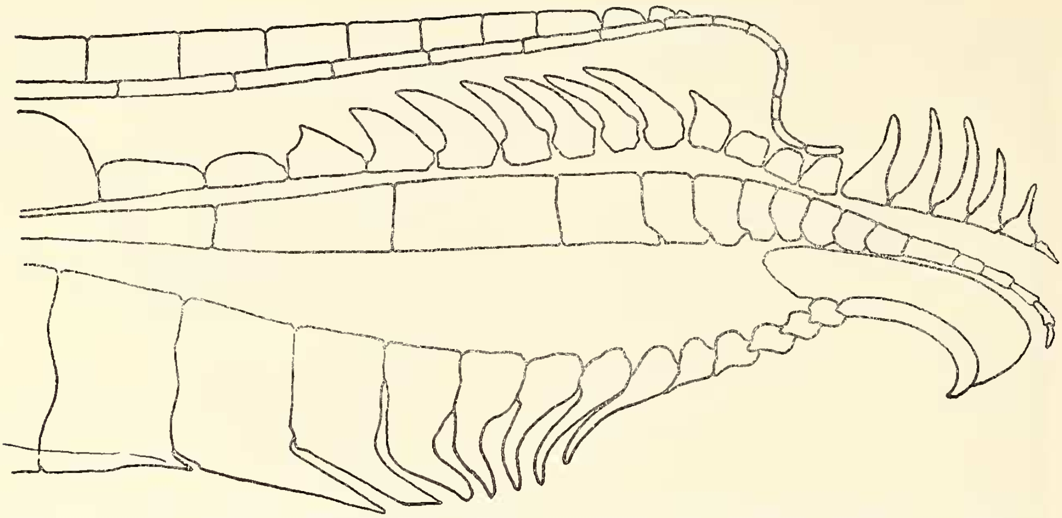
	Radioiodine-treated Fish			Untreated Controls
	Not Treated	KI-treated	Thyroid-fed	
Fish at beginning of treatments	25	12	18	25
Survivors at end of experiments[2]	11	9	15	22
Percent. Survival	44	75	83	88
Average age (days) first male seen	.	.	114	159
Males with sperm	0	1[3]	6	7
Males with mature gonopodium	0	0	6	7
Females with yolked oocytes	0	0	3	5
Pregnant females	0	0	1	0
Percent. histologically mature fish	0	11[3]	60	55
Average standard length (mm.)[4]	17.7	.	20.1	20.5
	21.8	22.0	.	24.5

[1]Each experiment included radioiodine-treated controls and non-treated controls, and the fish in each experiment were broodmates.

[2]Age of these fish at the end of the experiments was 6.1 to 6.8 months.

[3]This exceptional fish had a mature testis, but the anal fin was undifferentiated (see text).

[4]Two sets of comparative figures are given because all the measurements for the four groups could not be averaged and then compared, since the measurements were taken on photographs which varied slightly in magnification. Instead, means were taken for each of the groups within each experiment and these means were averaged for the groups shared by two or more experiments. For example: only experiments I and IV included KI-treated fish, but experiment I did not include thyroid-fed fish. Therefore the mean lengths of the KI-treated, the solely I^{131}-treated, and the control fish were averaged for these two experiments, but the thyroid-fed fish were omitted. This is represented in the second line of the comparison given for average standard length. The same method was used with experiments II, III and IV, excluding the KI-treated fish, and this comprises line 1. By this procedure, each experiment received equal weight, but the varying numbers of differently magnified fish in each treatment group in the experiments pooled was not allowed to distort the result.



TEXT-FIG. 4. The structure of the normal gonopodium of the male platyfish. In mature thyroid-fed, radioiodine-treated fish, in P^{32}-treated fish, and in controls, no deviation from the arrangement and numbers of the elements shown here was found (compare with photographs of gonopodia of variously treated fish in Pl. VII). Drawing by Dr. Donn E. Rosen.

Among the immature fish in these experiments, the thyroid-fed group included 4 fish with extremely undeveloped gonads, that is, males with no spermatogonial cysts and females with less than 5 differentiated ova. One such fish was found in the control group. These were among the smallest fish in each group. All other immatures in the thyroid-fed and control groups appeared to be progressing normally towards eventual maturity.

The pituitary glands of some of the thyroid-fed fish were included in volumetric analyses made on radioiodine-treated and untreated fish (Table 9). In two experiments which included thyroid-fed fish, the hypophyses of these proved to be as small, relative to the fish's body length, as those of I^{131}-alone-treated fish, and much smaller than those of the untreated controls. There was a morphological difference, however, from the radioiodine-treated controls: the neurohypophysis, although not measured other than by eye, appeared to be as large in the thyroid-fed fish as in their untreated controls, but the glandular portion was very much smaller (Text-fig. 3). In the solely I^{131}-treated fish the whole pituitary seemed to be smaller, just as the whole fish was smaller. Accuracy in projection and drawing was not good enough to make a sufficiently defined separation of the neural and glandular elements of the hypophyses to quantitatively verify this distinction. It seems reasonable to suppose that the thyroid-fed fish were receiving sufficient thyroid hormone to cause the thyrotrophic cells in their pituitaries to be reduced to a minimum in numbers and/or size, and activity, and thus for the glandular volume of the hypophysis to be considerably reduced.

The thyroid-fed fish were healthy and vigorous and as lively, or more so, than the untreated controls.

b. *Potassium Iodide Treatment:* The development of these fish was not noticeably improved over that of the fish treated only with I^{131}. There was, however, a definite improvement in the survival rate (Table 10), and they appeared to be healthier and livelier than the sluggish I^{131}-treated controls. Retardation of growth was not improved over the I^{131}-treated controls, and none became sexually mature, with the exception of one anomalous male (described above). Internal pathology was similar to that of the treated controls; vacuolated liver with its accompanying abdominal rotundity was found with almost equal frequency. Gonadal development was improved slightly, as judged by counts made of oocytes in females (average of 45+ in 4 KI-treated with a range of 20 to 84+; 14 in each of 2 radioiodine-treated controls) and by the number of males which had developed spermatogonial cysts (3 of 5 KI-treated; 1 of 4 radioiodine-treated controls) in experiments I and IV.

No evidence for the existence of thyroid tissue was found in 8 of 9 KI-treated, and in 5 of 6 radioiodine - treated controls. Because radioautography of KI-treated fish had been found to be unsuccessful, producing totally blank film strips (Baker, 1958b), only three of the present KI-treated fish were radioautographed. Some slight spotting was produced on these radioautographs, but only in the pharyngeal lining and gut areas. The remaining fish were examined section by section with particular care, and in all except one case no thyroid tissue was found in the pharyngeal, renal, splenic and other areas. The exceptions were a male KI-treated fish with a few regenerating pharyngeal follicles and a testis with the bare beginnings of spermatogonial cysts (experiment I), and a radioiodine-treated female with a single thyroid follicle in the kidney (Pl. II, Fig. 2) and an immature ovary containing 14 oocytes (experiment IV). The amount of thyroid tissue present in these two fish was apparently insufficient to improve their gonadal and visceral condition appreciably over that of other members of their groups.

The latter exceptional specimen was the only instance of finding renal thyroid tissue after the destruction of the pharyngeal thyroid at an early age. This represents an incidence of one out of 79 radioautographed fish, with or without regenerating pharyngeal thyroid tissue (experiment 1 excluded). With the exclusion of fish given replacement therapy, the incidence is one out of 42 radioautographed fish, or 2.4%; the incidence of renal thyroid tissue among untreated controls was 37 out of 50, or 74% (experiments 4-IV, in which sibling controls were kept). These results corroborate the ones previously presented by Baker (1958a) in support of an hypothesis that renal thyroid tissue in the platyfish arises as the result of cell migration from the pharyngeal area, rather than endogenously in other parts of the body.

4. *Radiation Control Experiments with Young Platyfish.*

Sixteen strain *BH* platyfish, in two groups, were treated with the amount of phosphorus-32 calculated to deliver whole-body irradiation equivalent to the average dose of radioiodine used. The conditions of exposure were similar.

Nine of the 16 treated fish survived until termination of the experiments, 109-178 days after treatment, and all of the 14 broodmate controls survived. One group of 5 fish was exposed to a P^{32} solution whose pH was not quite physiological (6.6), which may help to explain the high death rate seen among these fish, although the deaths did not occur until a month after treatment. As the single surviving P^{32}-treated fish in this group was a male, three female controls were discarded.

The over-all survivorship of the P^{32}-treated fish was 56%, but that of the 109-day, pH 7.5 group was 73% (8 out of 11 fish). This is not different from the 109-day survivorship shown for I^{131}-treated fish. Because of the small numbers of fish, no pattern of death distribution was recognizable, but it seems significant that there was no evidence for a bimodal mortality distribution such as that found for radioiodine treatment.

The average length attained by the P^{32}-treated fish did not differ from that of their controls (Pl. I, Fig. 4), *viz.*, 20.4 mm. for the P^{32}-treated (range 13.0 to 25.0 mm.), 21.1 mm. for the controls (range 18.5 to 24.5 mm.). Means were not corrected for numbers of fish of each sex, as these were as close to equality as possible in odd-numbered groups, being 5 males, 4 females among the treated fish and 6 males, 5 females among the controls. Upon fixation, at ages between 4.7 and 7 months, all except one fish in the treated and one in the control group appeared to be fully mature by external criteria. Moreover, the P^{32}-treated fish did not differ from the controls in body shape, coloration or general health and activity.

All of the fish were studied histologically. No abnormalities were found in the viscera of the treated fish, *i.e.*, liver, kidney, spleen and thymus were normal. Goitrous thyroids were found in 3 out of 9 of the treated specimens, and in 5 out of 11 of the controls. Renal thyroid tissue occurred in at least 6 of the treated fish and in 7 of the controls. It should be noted that the kidneys were not carefully searched; follicles were discovered in spot checks of the kidneys, implying that they were quite numerous in the specimens in which they were seen.

The only difference found between the P^{32}-treated fish and the controls was in gonadal development, primarily among males. Five of the 6 control males had fully mature testes, full of spermatophores, as well as completely differentiated gonopodia. In the testes of the 6th, spermatogenesis had begun but no spermatophores had yet appeared; the gonopodium was partially differentiated and was at a normal incomplete stage (Grobstein, 1953). Of the 5 treated males, 4 had fully normal mature gonopodia (Pl. VII, Fig. 3), but *none* had any testicular germ cells. Only the ducts were present, but these were complex in structure (Pl. VII, Fig. 5). One treated male had juvenile, simple ducts and an undifferentiated anal fin. Among the 5 control females, all had fully mature ovaries and two were gravid (Pl. VIII, Fig. 1); all 4 P^{32}-treated females also appeared to have completely mature normal ovaries containing fully yolked ova and young yolkless oocytes in all stages of maturation (Pl. VIII, Fig. 5). No counts of ova were made, but from the general microscopic appearance of the ovaries, there appeared to be no fewer in the P^{32}-treated female fish.

From these data it is concluded that P^{32} irradiation, equivalent to that received in the I^{131} treatment, produced none of the pathological or developmental effects noted after radioiodine treatment. The only effects were a very specific and distinctive action on the male gonad, which did not affect secondary sexual characters, and a similar total mortality rate to radioiodine treatment, up to 109 days post-treatment. The specific causes of mortality resulting from the two treatments is probably not similar, when the lower mortality rate of radioiodine- treated fish fed thyroid or KI-treated is taken into consideration.

5. *The Effects of Radioiodine Treatment in Adult Platyfish.*

a. *Survival:* Forty-six fish, all under one year of age, were injected with I^{131}. Ten of these died within two days after the injection; this is attributed to shock or infection and is not considered in relation to radiation damage or to thyroid condition. Two additional fish may also have died this way, but they were not recorded and are not considered here. Eleven more fish died within 8.5 months after injection; that is, ⅓ of the 34 remaining fish. Unfortunately, data on the mortality of the controls was poorly kept, but in Group 3, 3 out of 12 controls were recorded as dying within 8.5 months after the injection of their siblings. This suggests a somewhat higher death rate among the I^{131}-treated fish, but is by no means conclusive. Six out of 23 radioiodine-injected individuals (omitting those sacrificed when younger and those dying from the immediate effects of injection survived to an age of 27 months or more. At death, histologically visible thyroid was present in some of these old fish and absent in others.

b. *Gonadal and General Pathological Effects:* It is doubtful whether radiothyroidectomy (or near-thyroidectomy) had any profound effect on the gonads of these mature fish, at least up to 6.5 months after treatment. In Group 1, two of the three fish had fully normal mature ovaries. The third fish had given birth 22 days before she was radioautographed and contained no fully yolked ova; normally these should have been present soon after parturition (Tavolga, 1949). Thus a slight delay may be noticeable in this instance. In Group 2 (females and males kept together) young were born in the tank up to three months after treatment. Three females, fixed within 3.5 months of the injection, had normal ovaries and one was gravid; the single male sacrificed within this period had normal testes.

Among the female fish examined at longer intervals after injection (no males survived), there appeared to be some effect on the ovaries. These fish include 10 females from Group 3 and one from Group 2, sacrificed 8.5 to 19.5 months post-injection. The youngest 5 were noted to be "thin" when radioautographed. Only one of these had any functional-looking ova; this fish had a single basophilic, pre-yolk-deposition oocyte, and primitive gonial cells appeared to be proliferating from the lining of the ovarian cavity, in small balls resembling primary spermatogonial cysts in young males (Pl. VIII, Figs. 7, 8). Although the future function of these cells is unknown, the condition is suggestive of Essenberg's (1926) description of the development of testes from the epithelium of the ovarian cavity in sex-reversing females of *Xiphophorus helleri.* Essenberg's suggestion of a relation between depressed metabolism and sex reversal is also of interest in the case of the hypothyroid platyfish. In the other 4 fish, only fibrous atretic follicles were present. The 5 older fish from Group 3, which were 27.5 to 30 months old, included two with yolked oocytes, but only one of these had ova in earlier stages of development. The other three fish had ovaries with atretic follicles only (Pl. VIII, Fig. 6). Although the three sibling controls for these last 5 fish also had atretic follicles in their ovaries, they had many ova in various early stages of maturation as well. The single female from Group 2 was 28-29 months old and had an almost completely degenerate ovary with practically no visible germ cells. This fish exhibited several other pathological conditions (see below).

Vacuolated liver in varying degrees was found in 4 of the 18 I^{131}-injected fish, and ceroid-filled liver cells and/or ceroid-filled macrophages in the liver were seen in three additional fish. In one fish that died while in radioiodine tracer, the liver was decomposed too badly for its condition to be judged. Excessive peritoneal vacuolation was seen in 9 of the 16 fish that were freshly preserved.

Concretions were seen in the kidney tubules of 5 fish, and degenerate tubules in two, one being the extreme case described below. Lymphoid tissue in the kidneys appeared normal in all but the latter, and likewise the thymus glands appeared normal.

The pituitary glands of 11 of the 16 well-preserved fish showed a prominent overgrowth of the pale intermediate basophiles (Pl. VI, Fig. 6). This was found in all fish of the *Fu* strain. It also was found in all strain *163* fish fixed more than 8.5 months after injection, but not in strain *163* fish 38 days to 8.5 months post-injection.

This was a markedly higher incidence than in the young fish radiothyroidectomized by immersion, and is believed to be related to the incomplete degree of thyroidectomy in the injected mature fish, rather than to their age.

c. *Anomalies Noted:* From Group 2, in one male and one female, aged 12-13 months, a large mass of pathological tissue was found in the abdomen among the pancreatic and fatty tissue. In the male, this mass was attached to the normal testis (Pl. IX, Fig. 1). These masses were encapsulated and contained collections of whorled fibrous nodes. These nodes resembled, in part, the fibrous "nests" seen in various organs of other fish, but did not occur elsewhere in the bodies of these two specimens. A similar anomaly occurred in one 27.5-30-month-old female from Group 3.

In Group 1, a 15-month-old female had "nests" of fibrous tissue scattered throughout its body, including the dorsal pericardium and transverse pharyngeal muscles, the kidneys, liver, spleen and abdominal fatty tissue. None occurred in the ovary, heart and thymus areas. These "nests" of cells have been described in KI-treated platyfish with thyroid tumors in their kidneys by Baker (1958b) and in *Astyanax* by Rasquin & Rosenbloom (1954). They probably represent a reaction to injection or other bodily insult. It is unlikely that they are directly related to the I^{131} injection or to processes of thyroid degeneration, as the fish in which they were seen had been treated 3.5 months or more earlier.

The oldest fish in Group 2 (a 28-29-month-old female) was an extreme specimen. Its pathology included an almost total lack of lymphoid tissue (no thymus, shriveled kidneys and spleen) and an *extremely* highly vacuolated liver and abdomen, with excessive deposits of pigment in the spleen and kidneys, large necrotic cysts in the liver and a pituitary overgrowth of such proportions that it might well be classed as a tumor (Pl. IX, Figs. 3-7). There was no sign of hypertrophied thyroid, as might have been expected from the condition of other organs; instead the thyroid area was filled with fibrous tissue and masses of material which suggested thyroid, but which had flat margins and was stained a pale bluish color. The exact nature of this material is unknown, but the presence of thyroid cells is most unlikely. This fish appears to be the most extreme case of pathological hypothyroidism met with in this study. This fish also contained some of the "nests" of cells mentioned above, especially in subcapsular edematous spaces in the shriveled kidneys and in the necrotic cysts of the liver. The kidneys were deficient in glomeruli; many tubules seemed excessively small, others contained large concretions and had clear, "blown-up" cells that were unquestionably degenerate.[7]

None of these anomalies was found among the controls, but because of the small number and the relatively advanced ages of these fish, the peculiarities cannot definitely be related to hypothyroidism, except to surmise that such animals are very likely more prone to infection than normal similarly aged fish. A study of general senile degeneration has not been made on normal untreated *Xiphophorus maculatus,* but it is suspected that some of the effects noted here may be the result of a slightly premature appearance of such degenerative processes. Other effects appear to be definitely related to thyroid function, irrespective of age, *e. g.,* vacuolation of the liver.

In addition to the pathological anatomy, it should be noted that renal thyroid tissue was seen, without use of radioautographs, in all of the 6 control fish and in the spleens of three of them, but such tissue was not found or radioautographically suggested in any of the I^{131}-injected platyfish.

d. *Young Born after I^{131} Injection of Mother*: One female of Group 1 gave birth to young 19 days following the injection of approximately 80 μc. of I^{131}. Two of these young were radioautographed with the mother when they were 22 days old. One was found to be totally thyroidectomized, both by autographic and histological criteria. This was a female; the other was a male that had many thyroid follicles. These follicles, as is typical in very young fish, were made up of high basophilic cells. Both young seemed normal, except that the thyroidectomized female showed some vacuolization of the liver, and an aorta that seemed shrunken. Both young had undifferentiated gonads, although they definitely were sexable, and the female appeared to have large, clear oogonia at the cranial end of the oviduct. The testes of the young male fish were almost as well developed as those of some of the totally radiothyroidectomized fish 6-9 months old (Pl. VII, Figs. 6, 7). Since no normal young of equal age were sectioned, gonadal development was not controlled in these young fish.

According to the accepted gestation period of platyfish, *i.e.,* 22 days (Tavolga, 1949), the mother fish was injected when the young were

[7]The kidneys of this specimen were in an even more pathological condition than those of fish with the most extreme renal thyroid tumors described by Baker (1958b).

only three days in development. Since the thyroid gland does not appear until about the 8th day (Tavolga, 1949), enough radioiodine must have been retained by the yolk or the maternal tissues to affect the young thyroid when it first began to concentrate iodine, 5 days later. Another possibility is that the treatment slowed down the development of the young, so that they were born belatedly and were actually at the thyroid development stage when the mother was injected. It is known that most non-thyroid tissue and the yolk of platyfish ova do not retain iodine in protein-bound form (Baker, 1958a), but it is quite possible that *iodide* may be retained in such tissues for considerable periods. This instance suggests that sufficient I^{131} could be retained in the body of an adult fish to thyroidectomize both the adult, and later, young fish developing in its body. There was no possibility of any appreciable amount of I^{131} remaining in the aquarium by the time these young were born because of the number of times that the water had been changed since the time of injection. Thus, it seems probable that embryonic platyfish can be thyroidectomized *in ovarum* and still develop to a normal birth.

Discussion

1. *Radiothyroidectomy*.—Table 11 summarizes published attempts, to date, to extirpate the thyroid of teleosts using radioiodine. These few attempts have met with mixed success. It is possible that the differences between the activities of the thyroid found in various species of fish are responsible for the variety of results obtained (as an example of such differences, see the three species of *Fundulus* compared by Gorbman & Berg, 1955). It seems likely that less active thyroid glands, which both concentrate and release iodine and thyroxine or its relatives less rapidly, will receive more irradiation from a relatively prolonged dose of I^{131} than will very active thyroids. It is believed that the differences in the efficiency of radiothyroidectomy in the various strains of platyfish described here were at least partly due to genetic differences in iodine requirements and metabolism. Those strains that were more goiter-prone (or had less actively secreting thyroids?) seemed to be more readily radiothyroidectomized. Differences in the radioresistance of thyroid cells might also be a factor among different species or populations of teleosts.

The platyfish discussed here were probably

TABLE 11. SUMMARY OF REPORTS OF THE USE OF RADIOACTIVE IODINE IN THYROIDECTOMY OF TELEOST FISH

Author and Date	Species and Stage used	Degree of Thyroidectomy Obtained	Observations Made
LaRoche & Leblond, 1954	Atlantic salmon (*Salmo salar*), parr	Complete	No effect on growth; reduction of skin pigmentation
Baker *et al.*, 1955	Platyfish (*Xiphophorus maculatus*), young	Some complete	No effect on growth and sexual maturation[1]
Arvy *et al.*, 1956	Rainbow trout (*Salmo gairdneri*), parr	Incomplete	No effect on O_2 consumption
Fromm & Reineke, 1956	Rainbow trout, fingerlings	Complete	No effect on O_2 consumption
Fontaine, Y.-A., 1957	Eel (*Anguilla anguilla*), silver adult females	Complete	Decreased hypophyseal thyrotropin; no size difference in hypophysis; no TSH in plasma
Fontaine, M. *et al.*, 1957	Eel, adult silver	Presumed initially complete	Fall in O_2 consumption after 48 hours; later recovery presumed correlated with thyroid regeneration
Olivereau, 1957	Eel, small males; large females	Incomplete	Thyroid destruction and regeneration studied
Baker, 1958a	Platyfish, young	Many complete	Some pathological effects noted; effect on heterotopic thyroid development studied
Harris, 1959	Killifish (*Fundulus heteroclitus*), adult males	Mostly incomplete	No effect on salinity tolerance nor on growth or gonads

[1]These early published conclusions were mistaken and were corrected in the oral presentation of the material (see also Pickford, 1957). Complete results are embodied in the present paper.

more readily thyroidectomized by I^{131} than some of the fish used by other workers because they had been bred in an especially iodine-poor environment and because their iodine-to-thyroxine turnover was relatively slow. Despite the administration of thyrotropin prior to each of several I^{131} injections, Harris (1959) was unable to obtain complete thyroidectomy in most male *Fundulus*. Perhaps a more protracted period of exposure to I^{131} would be required if the turnover rate of iodine in these fish were relatively high within each post-injection period.

2. *Thyroid and Development.*—Various aspects of the function of the thyroid in fish have been thoroughly reviewed recently by Lynn & Wachowski (1951), Hoar (1957), Pickford (1957) and Leloup & Fontaine (1960). Papers that present evidence for thyroidal involvement in development and sexual function of teleosts, based on observations of non-experimentally treated fish, are included in these reviews. Some of these papers are omitted from discussion here. A synopsis of exclusively experimental observations on the effects of thyroid preparations and antithyroid compounds in the development of teleost fish is presented in Table 12. Many of the entries in this table have been more extensively summarized by Pickford (1957). Therefore the substance of the table will not be discussed here, except in a general way.

Among the more striking features to be noted in Table 12 is the heterogeneity of the results obtained with thyroid preparations and the relative homogeneity of those obtained with antithyroid drugs. One reason for this is probably the uniformity of dosage employed in the latter groups and the chemical uniformity of the drugs employed. Nearly all investigators agree that treatment with thiourea or thiouracil retards both growth and sexual differentiation in young fish, and brings about regression of gonads and loss of weight in adults. The effects of radiothyroidectomy of young platyfish observed by the author agree with this consensus, except that gonadal effects on adults were doubtful. LaRoche & Leblond (1954) reported that radiothyroidectomy had no effect on growth of salmon parr, but since histological criteria alone were used to determine the extent of thyroidectomy, it is suspected that the fish may not have been totally thyroidectomized. In some of the platyfish described here, it was found that a barely visible amount of thyroid tissue was sometimes sufficient to support sexual maturation and growth to normal adult size, and this barely visible tissue was found only with the aid of radioautography.

The results of treatment with thyroid derivatives run the gamut from retardation to enhancement of almost every feature of development. This variety in response is probably the result of the different methods of administration, types of preparations and dosages that were employed. In all instances, the fish undoubtedly were receiving abnormally high amounts of thyroid hormones, especially as their own thyroids were also present, even though their activity may have been inhibited. The occurrence of exophthalmia, abnormal gonopodial development, vascular abnormalitites and deformations of the head certainly cannot be considered as physiological, and it is hard to assess such peculiarities in terms of the normal function of the thyroid hormone. The most commonly described findings seem to be: slenderization in body shape; increased fin, skin and connective tissue development; silvering (increased guanine deposition) and reduced pigmentation in salmonoids; and exophthalmos. Yet even among these, there are reports describing the exact opposite, or the absence, of these features—sometimes in the same species. The effects on sexual development and over-all body growth are even more conflicting. Some of this disagreement may be related to the age of the fish tested, as well as to species differences in the coordination of thyroid function with developmental stages. Observations reported here suggest that reproductive processes in fully adult platyfish are relatively independent of thyroid function, just as growth, once completed, no longer can be generally influenced. Histological evidence suggested a sharp falling off of thyroid activity with age, after full attainment of mature body size, in this species.

Among young poeciliid fishes (including the guppy, platyfish and mosquito fish, *Gambusia*), increased growth rate, early male secondary sex differentiation, abnormal gonopodial development, increased fin length, and exophthalmia have usually been produced by treatment with thyroid derivatives. The results of thyroid-feeding of athyroid platyfish, reported here, concur with increased growth (increased to *normal*, that is), slightly accelerated male secondary sexual development, and some elongation of the caudal fin. They disagree in that gonopodial differentiation was completely normal in all cases and that no exophthalmia occurred. In comparison with controls, the proportion of mature fish was slightly higher at the conclusion of the experiments and only among the thyroid-fed, athyroid fish was a pregnancy found. It is believed that the normal development of these fish took place because they were given very little thyroid material. For example, the amount of dried

TABLE 12. SURVEY OF EXPERIMENTAL EVIDENCE RELATING THYROID FUNCTION TO GROWTH AND SEXUAL MATURATION IN FISHES[1]

Author and Date	Species and Stage Treated	Method of Treatment	Observations and Conclusions
A. *Effects of treatment with thyroid derivatives:*			
Terao, 1922	Goldfish (*Carassius auratus*)	Sheep thyroid powder fed, 1/25 of food	Depigmentation; stimulation of head growth
Blacher, 1927	Goldfish Crucian carp (*C. carassius*)	Thyroid gland fed	Depigmentation; exophthalmia
Sklower, 1927	Trout (species not given), fry	Thyroid fed in food (source not specified)	Slenderization of body shape
Zarski, 1927	Loach (*Misgurnus fossilis*) *Tinca vulgaris*	Beef thyroid fed or injected I.P.	No effect on histology of skin
Herzfeld *et al.*, 1931	Brook trout (*Salvelinus fontinalis*), hatchlings	Thyroxine by immersion, 0.002 gm./250 ml.	Acceleration of growth rate; darkening; spasms
Baumann & Pfister, 1936	Rainbow trout (*Salmo irrideus*), embryos	Thyroxine by immersion, solution 1:100,000	Acceleration of growth; vascular abnormalities, causing death
Krockert, 1936	Guppy (*Lebistes reticulatus*) young	Dried beef thyroid fed, 1.5-2.0 mg./day/fish	Enlargement of all fins; exophthalmia
Grobstein & Bellamy, 1939	Platyfish (*Xiphophorus variatus*), young	Desiccated thyroid fed, a "pinch" daily	Decreased growth rate; slender shape; all fins elongated; exophthalmia; precocious male sex differentiation, with abnormal gonopodial development
Landgrebe, 1941	Atlantic salmon (*Salmo salar*), parr	Ox thyroid extract injected 2× weekly (= 0.5 gm. fresh tissue)	Silvering; no gonadal effect
	Brown trout (*S. trutta*), adults	Same (= 1 gm. fresh tissue)	Silvering
	Eel (*Anguilla anguilla*), immature, yellow	Same, weekly	No effect on color or gonads
Hasler & Meyer, 1942	Goldfish, adults	1-6 mg. thyroxine injected I.P.	No effect on spawning
Svärdson, 1943	Guppy, young males	Thyroxine (method not specified)	Retardation of growth; earlier male maturation
Smith & Everett, 1943	Guppy, newborn	Thyroid powder fed; Synthetic thyroxine by immersion (8-15 drops/day of 2 mg./ml. sol. to 5-gal. tank)	No effect on growth in length or on male sex differentiation
Albert, 1945	Killifish (*Fundulus heteroclitus*), adults	Thyroxine; desiccated thyroid: various (unspecified) single doses injected	Exophthalmia not produced
Nigrelli *et al.*, 1946	Guppy, young, thiourea-treated	Mammalian thyroid powder fed, 0.2 gm. 2× weekly	Protected against retardation of growth and of male secondary sex differentiation, caused by thiourea

TABLE 12. SURVEY OF EXPERIMENTAL EVIDENCE RELATING THYROID FUNCTION TO GROWTH AND SEXUAL MATURATION IN FISHES[1] (*Continued*)

Author and Date	Species and Stage Treated	Method of Treatment	Observations and Conclusions
Vilter, 1946	Eel, leptocephalus ("glass eel")	Synthetic thyroxine by immersion, 1:1,000,000-1:50,000	Growth of stomach; increased pigmentation—but only when thyroxine concentration was raised progressively
Gerbilsky & Saks, 1947	Sturgeon (*Acipenser stellatus*), larvae	Thyroxine by immersion, 2×10^{-7}; 1×10^{-7}	Increased rate of development of bony plates
Zaks & Zamkova, 1947 (as summarized by Chambers, 1953)	Loach, embryos	Thyroxine by immersion, 1×10^{-7}; same plus 0.033% thiourea; thiourea alone	Increased bony scute development; increased resorption of yolk & external gills; change to adult-shaped head; gut secretory processes increased; retraction of pigment cells; iris pigmentation like adult. Thiourea plus thyroxine: *increased* the effects of thyroxine
La Roche, 1949 (abstract)	See La Roche *et al.*, 1950		
Robertson, 1949	Rainbow trout (*Salmo gairdneri*), parr, 2 years old, 9-10 inches long	Hog thyroid powder extract injected 3× weekly, doses increasing from 6.6 mg.; total dose 0.186 gm.	Silvering; increased guanine deposition
Smith, 1949	Coho salmon (*Onchorhynchus kisutch*), fingerlings	Na-thyroxinate by immersion, 1:1,125,000; desiccated thyroid fed	Slenderized shape; increased silvering
Hopper, 1950 (abstract)	See Hopper, 1952		
La Roche, 1950 (abstract)	See La Roche *et al.*, 1950; La Roche, 1951		
La Roche *et al.*, 1950	Atlantic salmon, parr	Na-thyroxinate injected; beef thyroid fed; thyroid extract fed 1:1 with beef liver	Pallor; endophthalmia (illusory—due to thickening of skin); deformation of head; loss of parr marks
Rizzo, 1950 a, b	*Gambusia affinis holbrooki*	No information given	Exophthalmia; hypertrophy of optic nerve; abnormal brain proportions; abnormalities in bones of skull and jaws; hypertrophy of choroid gland of eye
Buser & Bougis, 1951	*Gambusia affinis*, young	Thyroxine by immersion, 5-10 mg./liter	Exophthalmia; elongation of pectoral fins, no elongation of caudal fin
Hoar, 1951	Atlantic salmon, parr	Desiccated thyroid fed	Some increased silvering, but not smoltification
	Brook trout	Same	No silvering in 2 months
	Coho salmon, fingerlings, alevins	Same; also synthetic Na-thyroxinate by immersion, 1:1,125,000	Increased silvering not pronounced
	Coho fry	Na-thyroxinate 1:2,000,000	Pronounced silvering
	Chum salmon (*O. keta*), alevins	Same, 1:1,000,000	Pronounced silvering; thinning of skin; loss of scales; withdrawal of liver fat; slenderization of shape

TABLE 12. SURVEY OF EXPERIMENTAL EVIDENCE RELATING THYROID FUNCTION TO GROWTH AND SEXUAL MATURATION IN FISHES[1] (*Continued*)

Author and Date	Species and Stage Treated	Method of Treatment	Observations and Conclusions
Jones *et al.*, 1951	Zebra fish (*Brachydanio rerio*), embryos	Thyroxine; thyroid powder; by immersion, various concentrations	Retardation of morphogenesis and pigment formation
La Roche, 1951	Atlantic salmon, fry, parr, and smolt	Dried beef thyroid fed, 1:1 with diet	Increased dermal thickness
	Brook trout, yearlings	Same	Increased dermal thickness; increased internal organ connective tissue
Gaiser, 1952	Guppy, young	Thyroxine by immersion, 0.0001 per 100	Decreased initial growth rate; earlier onset of sexual differentiation in both sexes; no exophthalmia
Hopper, 1952	Guppy, young	Mammalian thyroid powder by immersion, 0.01%	Increased growth rate, especially in females (females 40-90% larger; males 20-30% larger, than controls); precocious abnormal differentiation of gonopodium in males
Ito, 1952	Goldfish, black & orange (2 fish)	"Thyroid hormone" by injection (no data on dosage)	No effect on pigmentation
La Roche & Leblond, 1952	Atlantic salmon, fry, parr and smolt	Thyroid extract fed 1:1 with liver; dried beef thyroid fed 75% of diet; 200 μg Na-thyroxinate injected intramuscularly 2× weekly	Some retardation of growth (weight, not length); thickening of integument and connective tissue—affecting head shape; pallor due to fewer pigment cells
	Brook trout	Beef thyroid fed 1:1 with liver	Similar
Müller, 1953	Goldfish	Synthetic thyroxine by injection I.P.	Young animals: melanophore expansion. Older animals: melanophore expansion at first, later depigmentation due to loss of melanophores
Smith *et al.*, 1953	Guppy, young, treated with 0.011-0.033% thiourea	Mammalian dried thyroid fed, 1:1 with food or by itself	No protection against retardation of sex differentiation and growth due to thiourea. Thyroid alone: slight delay in male differentiation? No growth effect
Dales & Hoar, 1954	Chum salmon, embryos	Na-thyroxinate by immersion, 1:2.5, 5.0 or 12.5 × 10^6	Speeded hatching; decreased body length; increased growth of body wall and pectoral fins (only); increased guanine deposition; exophthalmia
Gibson, 1954	Zebra fish, embryos	Thyroxine by immersion, 1-100/1,000,000	Same as Jones *et al.*, 1951; also stimulated heart rate
Honma & Murakawa, 1955	Chum salmon, larvae	Thyroxine by immersion, 1:1,000 & 1:1,000,000	Inhibition of weight increase; delay in absorption of yolk; broadening of head, protrusions on head; exophthalmos-like condition; elongated pectoral fins; poor development of body; pallor; silvering (excess guanine)

TABLE 12. SURVEY OF EXPERIMENTAL EVIDENCE RELATING THYROID FUNCTION TO GROWTH AND SEXUAL MATURATION IN FISHES[1] (*Continued*)

Author and Date	Species and Stage Treated	Method of Treatment	Observations and Conclusions
Egami, 1957	Medaka (*Oryzias latipes*), adult females, treated with testosterone and thiourea	Thyroxine by immersion, 1/6-1/18 mg./liter	Effects of thiourea mostly overcome by thyroxine treatment (see B. below)
Jones & Huffman, 1957	See Jones *et al.*, 1951; Gibson, 1954		
Langford, 1957	*Fundulus heteroclitus*, adults	Desiccated thyroid, 0.6 mg./day, or triiodothyronine, 0.1, 0.2, 0.5 or 1.0 μg. injected daily	Marked exophthalmia produced
Bjorkland, 1958	Goldfish, young	Thyroxine, triiodothyronine injected I.P. in "small doses" Parrotfish thyroid injected I.P.	Epidermal thickening; pigmentary paling; increased growth in weight and length No morphogenetic or growth effect (immune reaction?)
Matty *et al.*, 1958	*Sparisoma squalidum*, adult female, young *Scarus croicensis*, adults *Bathystoma aurolineatum*, adults (?)	L-thyroxine, 10 or 500 μg injected daily; triiodothyronine, 5 μg daily	Marked exophthalmia, independent of dose
Stolk, 1959	Swordtail (*Xiphophorus helleri*) × platyfish (*X. maculatus*) hybrids, young	Thyroxine fed mixed in food	Increased growth rate; inhibition of melanotic tumor development
Honma, 1960	Chum salmon, larvae	Thyroxine by immersion, 1:1,000,000	Acceleration of metamorphosis, silvering, increased height of skull, elongation of opercular bones, hyperplasia of orbital connective tissue, inhibition of yolk absorption and differentiation of some viscera, retardation of growth rate
Hopper, 1961	Guppy, young	Thyroid powder fed 50 mg./3000 ml. H_2O/diem (no. of fish per tank not specified)	No effect on growth rate or sexual differentiation
B. *Effects of treatment with antithyroid drugs:*			
Goldsmith *et al.*, 1944	Platyfish × Swordtail hybrids, young	0.033% thiourea by immersion	Retardation of growth and failure of sex differentiation
Nigrelli *et al.*, 1946	Guppy, young	0.03% thiourea by immersion	Inhibition of sex differentiation
Zaks & Zamkova, 1947	Loach, embryos	Thiourea 0.033% by immersion	No effect up to 26th day of development

TABLE 12. SURVEY OF EXPERIMENTAL EVIDENCE RELATING THYROID FUNCTION TO GROWTH AND SEXUAL MATURATION IN FISHES[1] (*Continued*)

Author and Date	Species and Stage Treated	Method of Treatment	Observations and Conclusions
Frieders, 1949	Platyfish, Black molly (*Mollienesia latipinna*), Blue Acara (*Aequidens latifrons*), Blue Gourami (*Trichogaster trichopterus*), all young	0.05% thiourea by immersion; 0.0025% phenyl-thiourea by immersion	All died Retardation of growth; depigmentation (Blue Gourami: growth decreased at first, but after 5 weeks it *increased* over the controls)
Goldsmith, 1949	Platyfish × Swordtail hybrids, young	0.03% thiourea by immersion	Retardation of growth; inhibition of secondary sexual characters
Iakovlova, 1949 (as summarized by Pickford, 1957)	Sturgeon (Sevriuga), larvae	Thiourea 0.033% by immersion	Inhibition of growth and scute development; retention of larval teeth
Smith, 1949	Chum salmon, alevins Sockeye salmon (*O. nerka*), alevins	0.36% thiourea by immersion	Retardation of growth and yolk usage; deformities observed; no effect on silvering
Hopper, 1950 (abstract)	See Hopper, 1952		
Leloup & Olivereau, 1950	*Dentex vulgaris*	Thiourea 0.5 mg./kg. injected intramuscularly daily	Exophthalmia developed after 2 injections & became accentuated after further injections; fish soon died
Jones *et al.*, 1951	Zebra fish, embryos	Thiouracil by immersion, various concentrations	Enhancement of morphogenesis & pigment formation
Barrington & Matty, 1952	Minnow (*Phoxinus laevis*), adults	0.1% thiourea by immersion	Arrest of spermatogenesis and oocyte growth; inhibition of external sexual characters
Gaiser, 1952	Guppy, young	0.03% thiourea by immersion	Growth and sex differentiation retarded—no females matured in 90 days
Hopper, 1952	Guppy, young	Thiouracil 0.03% by immersion	Females 5-15% smaller than controls; male differentiation failed in most cases—abnormal gonopodial differentiation when seen
Vivien & Gaiser, 1952	Guppy, young	0.03% thiourea by immersion	Retardation of growth and sexual development in both sexes
Warner, 1952	Brown trout, embryos, fry	0.033% thiouracil by immersion	Delay in yolk resorption and slight delay in hatching; impaired viability; no marked growth effect; sluggishness
Chambers, 1953	*Fundulus heteroclitus*, adult males	Thiourea, 0.5 mg./gm. body weight injected 3× weekly, reduced to ½ after 4 weeks	Loss of weight; retardation of growth; regression of testes; enlargement of liver—enlargement and vacuolization of hepatic cells, depletion of liver glycogen and fat; loss of appetite; high death rate
Scott, 1953	Zebra fish, young	0.33% thiourea by immersion	Growth retarded; gonad development retarded in youngest group

TABLE 12. SURVEY OF EXPERIMENTAL EVIDENCE RELATING THYROID FUNCTION TO GROWTH AND SEXUAL MATURATION IN FISHES[1] (*Continued*)

Author and Date	Species and Stage Treated	Method of Treatment	Observations and Conclusions
Smith *et al.*, 1953	Guppy, young	0.011-0.033% thiourea by immersion	Retarded growth and sex differentiation
Barrington, 1954	Minnow, adults	Thiouracil (no details given)	Completion of spermatogenesis, induced by artificial light, not affected
Dales & Hoar, 1954	Chum salmon, embryos	0.05, 0.1 and 0.2% thiourea by immersion	Reduced growth rate; slight delay in yolk resorption; decreased guanine deposition; no effect on pigmentation
Frieders, 1954	See Frieders, 1949		
Gibson, 1954	Zebra fish, embryos	Thiouracil, 1:500 to 1:100,000	Same as *Jones et al.*, 1951
Sembrat, 1954	Brown trout, embryos	5% methylthiouracil-Na by immersion	Absence of scale development; thinness of skin; increased pigmentation
Fortune, 1955	Guppy, young Minnow, hatchlings	0.05% thiourea by immersion	No effect on growth in either species
Honma & Murakawa, 1955	Chum salmon, larvae	Thiourea 1:3,000 by immersion	No color change; immature character of body form retained
Sembrat, 1956	Brown trout & guppy, young	0.05-0.1% methylthiouracil-Na, by immersion	Inhibition of scale development; thinner skin; inhibition of growth in both species
Egami, 1957	Medaka, adult females, treated with testosterone	Thiourea by immersion, ½-2.0 gm./liter	Pallor; slenderization; marked inhibition of formation of male fin processes in testosterone treatment
Honma & Murakawa, 1957	Goldfish, larvae	Thiourea by immersion, 1:3,000	Inhibition of growth (weight and linear dimensions); inhibition of guanine deposition in scales & of decolorization; bent vertebral column
Grosso & Charipper, 1958	Guppy, embryos	Thiourea by immersion of mother in 0.04% solution	Inhibition of ovarian maturation in adult; decrease in productivity; heterotopic thyroid in newborn young
Pflugfelder, 1959	Guppy	$KClO_4$ by immersion, 0.05%	Inhibition of sexual development
Stolk, 1959	Swordtail × Platyfish hybrids, young	Thiouracil fed, mixed in food	Decreased growth rate; enhancement of melanotic tumor development
Honma, 1960	Chum salmon, larvae, Goldfish, larvae	Thiourea by immersion, 1:3,000	Inhibition of differentiation, decrease in growth rate, abnormal calcium metabolism (reflected by abnormal bone development), increase in carotinoid pigments in goldfish, inhibition of decolorization in salmon
Kajishima, 1960	Goldfish, embryos	Phenylthiourea 0.05% by immersion	Development retarded, body shortened & deepened; exophthalmia; complete inhibition of differentiation of melanin granules in eye & body; xanthophores & guanophores degenerated 2 weeks post-hatching

[1]This table excludes observations made on metabolic rate, migration, osmotic regulation, regeneration and pituitary cytology.

thyroid initially given (as food), if dissolved throughout the water, would have been approximately 0.0016%; this eventually was doubled but not until the fish had grown considerably larger. This was one-sixth of the concentration used by Hopper (1952) for young guppies (0.01%), and when increased was still only one-third of his dosage. Without chemical assays, it is hard to compare doses of dried thyroid with doses of pure thyroxine, but probably many of the latter were excessive. For example, the 5-10 mg./liter dose of Buser & Bougis (1951) seems to be an extremely high concentration of thyroxine, since it is almost as high as the dried thyroid concentration first cited, and desiccated thyroid certainly is composed largely of materials other than thyroxine itself. That this dosage produced exophthalmia seems not surprising.

In studies on the effects of antithyroid drugs on fish, darkening has been noted: *e.g.*, as a result of the increase in pigmentation (Sembrat, 1954), or a decrease in guanine desposition in the scales, which then revealed the underlying pigment (Dales & Hoar, 1954). Although darkening was noted among the radiothyroidectomized fish studied here, the effect was not analyzed.

3. *Thyroid Activity and Liver Function.*—According to Spellberg (1954), fatty liver in man has been associated with hyperthyroidism, as well as with "anterior pituitary hyperfunction"—among numerous other causes. The first observation differs from experimental results in other mammals. Chaikoff *et al,* (1948) found fatty liver with fibrosis in thyroidectomized dogs after 193 to 799 days, and McClosky *et al.* (1947) noted fatty livers, as well as iron-pigment deposits in the spleens and livers of cats fed thiouracil for 7-8 months. Thirty days of thyroid-feeding reduced fat in the livers of normally fed rats, and protected rats on lipotrophic diets from the development of fatty liver (Leathem & Howell, 1950). These authors also noted that thiouracil, fed for 35 days, caused an increase in liver weight and cell size, without change of fat content. Similar observations were made on rats treated with thiourea up to 60 days by May *et al.* (1946); they found no amelioration of thiourea effects by simultaneous thyroid treatment. Leblond & Hoff (1944) also found that thiouracil-feeding of rats for 21 days led to an increase in liver weight, but that a *decrease* occurred in thyroidectomized animals. No chemical or histological analyses were presented. Sellers & Wen You (1951) found that thyroid hormone influenced the site of fat deposition in the liver of rats. In thyroid deficiency, fat tended to be deposited intracellularly throughout the liver, but in the normal, and especially in the hyperthyroid animal, fat accumulated in the central part of the liver lobule and extracellular fatty cysts were formed. Sellers & Wen You suggested that the protection afforded by propylthiouracil against experimental cirrhosis might be due in part to the reduction of thyroid hormone level, which shifted the pattern of fat deposition from extracellular to intracellular. Finally, Goldberg *et al.* (1950) found no persistent pathological changes in non-cervical tissues, *e.g.*, the liver and kidneys, of radiothyroidectomized rats up to 8 months after treatment.

In fish, only a few observations appear to have been made on the liver in relation to thyroid function, and these are not altogether in agreement. Hatey (1950) reported that thiourea treatment of carp (*Cyprinus carpio*) by immersion led to a decrease in liver size and liver glycogen within 3.5-11.5 days. Chambers (1953) also found a depletion of liver glycogen in *Fundulus heteroclitus,* treated with thiourea by injection for 6 weeks, but this was accompanied by an increase in liver size. Liver cell size was increased and fat content decreased. Pickford (1952) noted a decrease in liver size of hypophysectomized male *Fundulus heteroclitus* treated with thyroxine, but the difference from the controls was not considered significant. The observations of Chambers and Pickford agree with the results obtained for mammals with respect to organ and cell size, but both Chambers and Hatey found glycogen level to be affected in the opposite direction than that seen in rats (Leathem & Howell, 1950; May *et al.,* 1946). On the other hand, Fontaine *et al.* (1953) observed a decrease in liver glycogen after long-term, high dosage thyroxine treatment, at high temperatures, of eels (*Anguilla anguilla*) and rainbow trout (*Salmo gairdneri*), an observation which agrees with the mammalian results. On the basis of these results, these authors hypothesized that the drop in liver glycogen seen in smolts might be at least partly due to thyroid hyperfunction.

Liver size, histology, and fat and glycogen reserves of teleosts differ in the two sexes, and also vary during different stages of the breeding cycle (Olivereau & Leloup, 1950; Immers, 1953; Clavert & Zahnd, 1956a, b; 1957; Zahnd, 1959). In female poeciliids the liver/body size ratio was larger than that of males, but it decreased during the development of the egg yolk, and fat reserves were depleted (Clavert & Zahnd, 1956a). In inactive females and in males, fat reserves were large. Female hormones given to such fish of either sex induced liver changes parallel to those seen in females during vitellogenesis (Clavert & Zahnd, 1957). Tavolga (1949) found that certain female sex hormones when fed, exerted an opposite effect on the livers of platyfish. Liver cells became vacuolated and fatty changes took place—apparently in both sexes. Egami (1955) reviewed the Japanese literature reporting sex and cyclic differences in the liver of fishes. He found in *Oryzias* that the liver is larger in females and has larger cells. Implantation of estrone pellets into sexually active males led to liver changes in the female direction, but in inactive males there was no effect. Implantation of testosterone pellets or ovariectomy of females caused liver changes in a male direction, but more slowly than the changes in treated males. In sticklebacks (*Gasterosteus aculeatus*) and minnows (*Phoxinus laevis*), glycogen storage was greater in females, but it was largely located in the ovaries (Immers, 1953). Its changes were not regarded as responsible for the fluctuations of liver weight occurring during the sexual cycles. Oguro (1956) found the liver of female sticklebacks to be larger than that of males; there was more fat in the male liver, its cells were larger, its cells contained multiple nucleoli, in contrast to single nucleoli in female liver cells, and the mitochondrial morphology differed in the two sexes. Estrogen injected into males caused liver changes in the female direction.

Although the vacuolation of the liver in the radiothyroidectomized platyfish here described could have been due alternatively to accumulation of water, glycogen or fat—all of which could have presented the same picture after the solvent treatments used in preparation of slides —the histological picture was not like any of the illustrations offered by the above authors. Sexual inactivity, although a cause of fat or glycogen deposition in the liver, did not lead, in the reports cited above, to histological appearances even remotely approaching the extreme degree of vacuolization seen in thyroidectomized platyfish. Moreover, normally maturing, incompletely thyroidectomized platyfish developed as severely vacuolated livers as immature, totally thyroidectomized specimens. Therefore it may be concluded that the role of sex hormones in this instance is of negligible importance, in contrast to that of thyroid hormone.

The vacuolated livers in the thyroidectomized and hypothyroid platyfish, if they do represent fatty livers, developed under conditions which accord with the results presented by Chaikoff *et al.*, and the absence of vacuolated liver in thyroid-fed thyroidectomized platyfish is in line with the findings of Leathem & Howell. These mammalian results, and others summarized above, suggested that the presence or absence of thyroid hormone might have a different action on liver physiology than treatment with antithyroid drugs. Only the cats of McClosky *et al.* exhibited fatty liver after chemical thyroidectomy. This experiment was, however, the only one in which either mammals or fish were given antithyroid drugs for more than 60 days. Chambers observed ceroid in the livers of all *Fundulus* that had been thiourea-treated for 6 weeks, and the present author found ceroid in the livers of platyfish thiourea-treated for 10 weeks. Furthermore, vacuolated livers with ceroid were found by the author in platyfish treated with thiourea for 18 weeks. If ceroid is a product related to neutral fat,[8] its over-accumulation might precede and herald the onset of fatty liver. The development of fatty liver might require more prolonged antithyroid treatment than most of these experiments encompassed. The data reported here on thyroidectomized platyfish, as well as on thiourea-treated platyfish, show that vacuolated liver developed gradually over a period of many months (with one exception, vacuolated liver was not found in radiothyroidectomized fish examined prior to 60 days post-treatment). Extensive ceroid deposits found in several specimens were tabulated as equivalent to vacuolated liver.

The observations of Sellers & Wen You may reconcile the above-cited association between fatty liver and hyperthyroidism in man with the experimental results on other mammals if, in the human cases, the excess fat were present extracellularly. In the thyroidectomized and thiourea-treated platyfish the vacuolation was certainly intracellular, so that no conflict appears here. The failure of Goldberg *et al.* to find any pathological changes in long-term-thyroidless rats alone appears unreconcilable with other observations. Only genetic differences come readily to mind in explanation of this inconsistency.

[8]Ceroid is a clear yellow pigmented material which first was described in experimentally-produced liver cirrhosis of rats (Lillie *et al.*, 1941; Edwards & White, 1941). Its histological appearance and histochemical characteristics were described by Endicott & Lillie (1944); among these are insolubility in alcohol and ordinary fat solvents, and non-stainability by haematoxylin-eosin or Masson's trichrome (used exclusively in the experiments reported here). The identification of the material seen in the livers of the author's radioiodine-treated fish was first suggested by Chambers' (1953) description of ceroid in the livers of *Fundulus* treated with thiourea. Endicott & Lillie suggested that ceroid might either be a derivative of neutral fat or a separate product of abnormal liver metabolism. Ham (1952) described ceroid as an oxidation product of unsaturated fats.

It seems clear, at any rate, that in platyfish the thyroid hormone is deeply involved in liver metabolism, and that lack of it leads to excessive accumulation of ceroid, and probably of neutral fat.[9]

4. *Thyroid Function and Pituitary Development.* —Volumetric studies on the pituitaries of thyroidectomized and normal platyfish suggested that the glandular portion of the hypophysis was sharply reduced in thyroid-fed, athyroid fish, whereas the neural portion developed normally. In solely radiothyroidectomized fish, the entire pituitary appeared to be subnormally developed relative to the size of the animal. This was somewhat surprising, as lack of thyroid hormone might be expected to lead to increased thyrotrophic function on the part of the pituitary, and possibly thereby to an increase in over-all pituitary size. Control platyfish with hypertrophied thyroids were often found to have large ventral basophilic overgrowths of their pituitaries. Enlarged glandular portions of the pituitary also were found in goitrous teleosts by Stolk (1956a,b). Increases in cell number or size among pituitary basophils have been described in antithyroid drug-treated teleosts by Leloup & Olivereau (1950), Scott (1953) and Sokol (1957), and an increase in the number of eosinophils by Vivien & Gaiser (1952). Without specifying cell type, Honma & Murakawa (1955) stated that cells of the transitional lobe of thiourea-treated salmon larvae were enlarged. However, none of the latter authors mentioned a gross increase in the size of the whole pituitary. Atz (1953) and Barrington & Matty (1955) described degranulation of thyrotrophs in the pituitaries of antithyroid drug-treated fish, but did not mention increase in cell number or cell size.

The excessively small pituitaries of the radiothyroidectomized platyfish may have been the result of a general exhaustion of the several pituitary cell types in a futile effort to respond to a variety of physiological deficiencies triggered by long-term athyroidism. Severinghaus (1942), as quoted in a review by Maqsood (1952, page 304), stated that early thyroidectomy in mammals may inactivate the pituitary to the extent that a virtual hypophysectomy is produced. Maqsood cited the findings of several other workers that support this statement. The small size of the glandular portions of the pituitaries of thyroid-fed, athyroid fish, on the other hand, was probably due to inactivity, at least on the part of thyrotrophs, for their function was no longer demanded for normal physiological function of the whole animal, owing to the illusory production of an excess of product by the target organ.

5. *Thyroid Function and Behavior.* An increase in sustained activity after thyroxine treatment and a decrease in activity following thiourea treatment were observed in goldfish and salmonoids by Hoar *et al.* (1952) and Hoar *et al.* (1955). The studies of Woodhead (1959) have shown the importance of thyroid gland activity in the long migrations made by sexually immature, as well as mature, cod (*Gadus callarias*). Many years ago, Marine & Lenhart (1910) noted the sluggish behavior of hypothyroid (goitrous) trout, and they also noted that such trout died more readily than normal fish when taken from the water. Other examples of sluggish behavior after antithyroid drug treatment may be found in Table 12. Although the behavioral observations made on radiothyroidectomized platyfish in this paper were casual, it seems appropriate to note that the activity pattern was in accordance with detailed studies such as those cited, and that greater fragility, as noted by Marine & Lenhart, was found.

6. *Radiation Effects.*—Swelling and hyalinization of blood vessel walls in the thyroid area following moderate and high dosages of radioiodine was noted in adult male rats by Goldberg *et al.* (1950). Their description applies also to the effects seen on the aorta of radioiodine-treated platyfish. Similar in both rats and fish was the fibrous tissue replacement of thyroid tissue, sometimes in whorled form. The process of thyroid destruction in rats and fish followed parallel courses which were characterized by the swelling of the epithelial cells of the follicles, the loss of dense colloid, the disorganization of follicle structure, and the final disappearance of all thyroid tissue, leaving the stromal framework to be infiltrated by fibrous elements.

Most work on the effects of whole-body irradiation on fish has been done with either embryos or mature animals, rather than on young, growing fish equivalent in development to the platyfish studied here. Bonham *et al.* (1948) studied the effects of x-rays on fingerling Chinook salmon (*Onchorhynchus tschawytscha*). After 12 weeks, growth in length was affected only in fish that had received 1,000 r. or more. The cumulative death rate in fish receiving more

[9]Increased deposition of iron-containing pigment in liver and spleen was among the effects produced in mice that were fed thiouracil on a long-term basis by Dalton *et al.* (1946). This was noted also by McClosky *et al.* (see above) in their thiouracil-fed cats. Although this feature was not systematically studied, it is the author's impression that excessive brown or black pigment deposits were present in the spleens, though not the livers, of thyroidectomized platyfish, and that such deposits also were more frequent in the kidneys, as compared with controls (see Pl. IX, Figs. 4, 5).

than 250 r. was significantly higher than in controls. In fish receiving 500 r. or more, the renal hematopoietic cells were reduced in the first two weeks, with recovery by the 4th or 5th week in survivors of 500-1,250 r. The gonads were not studied. Welander *et al.* (1948) found somewhat greater sensitivity in embryonic and larval Chinook salmon than in the older fish. The lowest dose affecting growth in length was 500 r., and the lowest dose affecting hematopoietic tissue was 250 r. At 500 r., neither mortality nor weight was significantly different from the controls. Pigmentary development was affected only in fish receiving 1,000 r. or more. This also was true of other indices of development, such as eye size and gill growth. Recovery of hematopoietic tissue occurred in fish receiving 1,000 r. or less. Little or no damage to renal tubules and glomeruli was found in the latter groups. Splenic damage paralleled that of the renal hematopoietic tissue in dose effect and recovery. The gonads were retarded by all doses (250 r. and up), but nevertheless, in those receiving 250-1,000 r., they were progressing to definitive sex cell stages, 93 days after irradiation. Other organs examined were not affected by doses of 1,000 r. or less.

In mammals, including man, the hematopoietic tissue is especially sensitive to radiation (Tullis, 1959). At LD_{50} or higher doses (gamma radiation from atomic explosions), hematopoietic tissue was destroyed very rapidly in the lymph nodes and bone marrow, less rapidly in other lymphoid tissue. However, Tullis noted that the reticular (stem) cells are among the most radioresistant in the body; recovery of hematopoietic tissues began within about 4 weeks in survivors. The liver and kidneys were not found to be importantly affected. (Tullis mentioned that "Reversible accumulations of sudanophil fat in the liver have been noted in many animal species," but gave no specific references on this point). Ovaries were noted to be more radioresistant than testes; primordial ova are referred to as being among the most radioresistant of cells. Interstitial cells of the testis also were not noticeably affected.

Metcalf *el al.* (1954) found that in rats given 550 r. whole-body x-radiation, lymphoid degeneration in lymph nodes, spleen and bone marrow occurred, but returned to normal in 17-40 days. Spermatogonia, after destruction, reappeared in 20-40 days. There was no injury to the liver or kidneys.

Lushbaugh (1957), in a review, states that the normal liver is resistant to radiation up to 3,400 r. in mouse, rat and man, and that 20,000 r. did not affect liver regeneration in rats. The pancreas is also radioresistant, requiring more than 2,500 r. for visible effects.

The effects of I^{131} treatment of young and adult platyfish were similar in some respects to those reviewed above, resulting from other forms of irradiation. In particular, the hematopoietic reduction found was characteristic of radiation damage in other animals. However, the persistence of the condition, sometimes acutely, in survivors for 4-8 months after irradiation apparently is anomalous. Moreover, hematopoietic tissue in platyfish similarly treated with P^{32} in equivalent r. dosage (720 r.) was normal 3-4 months after exposure. These observations indicate that athyroidism or extreme hypothyroidism may prevent normal repair of hematopoietic damage resulting from radiation. This is borne out by the improved condition of this tissue in the thyroid-fed, radiothyroidectomized platyfish. The effect of the thyroid here, as in other instances, may not be tissue specific, but merely a reflection of a general positive effect on cell growth.

Although fatty liver apparently may occur transitorily in various species after radiation, this does not seem to be a commonly noted effect. When the P^{32}-treated platyfish, whose livers were quite normal, are considered in relation to the I^{131}-treated fish, the evidence favors the interpretation that the high incidence of severe liver vacuolation found was related entirely to thyroid insufficiency and was independent of radiation. Again, the livers of the thyroid-fed, thyroidless fish were completely normal, which further substantiates this assumption.

Growth retardation in young salmon, although a definite effect of radiation, was not found to be significant at 720 r., the dosage level received by young platyfish treated with I^{131} or P^{32}. There was no effect on the body length attained by P^{32}-treated platyfish, nor were their body proportions noticeably different from those of the controls. Moreover, the thyroid-fed athyroid platyfish were normal in body length and shape; in fact, a slight growth enhancement appeared in these fish, in the form of elongation of the caudal fin. The retardation of growth found in the solely radiothyroidectomized fish therefore may be attributed to the lack of thyroid hormone.

No sign of increased pigmentation, similar to the roentgen - pigmentation of goldfish (Ellinger, 1940), was seen in P^{32}-treated platyfish, nor were any pigmentary differences from controls noted in thyroid-fed I^{131}-treated fish. A generalized darkening was noted in many of the exclusively I^{131}-treated platyfish, but this was of the normal speckled pattern found in all fish of the strain employed and was not especially prominent on the head. This probably was a minor effect of the lack of thyroid hormone (see above).

The effects of treatment with I^{131} or P^{32} on the gonads of young platyfish were dissimilar, although both were adverse. Among solely radioiodine-treated fish, only one developed a gonad of mature appearance. In this male specimen, however, the gonopodium failed to differentiate. A single similar male occurred among I^{131}-treated, KI-treated fish. Apart from these exceptions, the failure of germinal and duct elements alike to develop beyond juvenile stages in thyroidectomized fish of both sexes stands in sharp contrast to the normal sexual differentiation seen in most thyroid-fed, athyroid fish. From these observations, together with those made on P^{32}-treated platyfish (discussed fully below), it may be concluded that the thyroid plays a powerful role in normal sexual maturation and that radiation effects were negligible.

The complete destruction of the germinal cells in the testes of P^{32}-treated males, together with normal gonadal duct development, confirms the observations of Vivien (1953a, b) on P^{32} treatment of immature and adult male guppies (*Lebistes reticulatus*) and swordtails (*Xiphophorus helleri*). That normal male secondary sexual differentiation was found also agrees with Vivien's results, and there seems no question that the sex cells themselves have no endocrine role in the development of these male poeciliids. With respect to females, P^{32} treatment of young platyfish had no readily discernible effect on ovarian development. Fertility was not tested, since the only males made available to these females were their sterile brothers just described. The ineffectiveness of P^{32} treatment of female platyfish is in disagreement with Vivien (1953b), who found an equal amount of germ cell destruction in both sexes. This difference is particularly surprising when doses are compared. The platyfish were treated with 1.7 mc. P^{32} for 48 hours, to give a total dose of 720 er. Vivien treated his fish, maximally, with 200 μc/liter of P^{32}, which was left to decay throughout the treatment of 130 days, fish removed to clean water after 8 days being no less affected. Using formulas given by Glasser *et al.* (1952), we calculate Vivien's procedure to give a total dose of 175 er. (or less, if all the P^{32} had not decayed in 130 days). This is much smaller than the dose given to the platyfish, yet it had a much more profound effect in the females. Two possible, alternative explanations might be, the chronicity of Vivien's treatment, or species differences in the fish. The latter is not likely, since Vivien used fish of two different genera with similar results, and one of his species is congeneric with the platyfish. The first alternative also seems unlikely in view of certain observations made on mammals, which are briefly summarized below.

Long-term gamma irradiation of mice at various dose rates led to observations showing certain similarities, but also profound differences from both Vivien's results and our own. Deringer *et al.* (1954) found that male mice given 1,100 r. at rates of 4.4 r. per 8-or-24-hour day bred normally, while those sterilized by higher total doses (1,760 r.) at the rate of 8.8 r. per 24-hour day recovered their fertility. The fertility of female mice given less total irradiation than males (770 or 880 r.) at the equal rates of 4.4 or 8.8 r. per 24-hour day, or 8.8 r. per 8-hour day, was reduced and some became totally sterile. Unlike males, females sterilized by higher total doses did not recover their fertility. Thus, the reproductive capacity in female mice was reduced or destroyed by doses of radiation which either did not affect male mice, or from which males recovered. Eschenbrenner & Miller (1954) published similar findings on mice and emphasized that inhibition of spermatogenesis was correlated with dose rate, not total dose. They found that interstitial tissue was unaffected up to 5,000 r. total dose. In guinea pigs, the male germ cells were similar in radiosensitivity to those of mice, but the ovaries were highly radioresistant. The latter was also true of rabbits.

The observations on the radioresistance of mammalian testicular interstitial cells agree with all data on P^{32}-treated fish, and also with Vivien's (1952) earlier observations on the effects of x-rays on the testes of swordtails. However, the results with mice are dissimilar to those of the author for platyfish. In the mouse, at a given dose rate, the ovaries of the females were more radiosensitive than the testes of males, but in the platyfish the females were more resistant to radiation damage. Platyfish thus seem to be more similar to the guinea pig or rabbit. In Vivien's experiments, the initial dose rate would have been 8.4 r. per 24-hour day, which was a rate that sterilized female mice, but was a very much lower rate than that given the platyfish (360 r. per 24-hour day). Yet the ovaries of the platyfish suffered no visible degeneration. Thus, the longer term of exposure of Vivien's fish to P^{32} irradiation, although the most likely factor in causing the discrepancy in results on female fish, might be ruled out on the basis of dose-rate considerations.

The observations of Samakhvalova (1935)

and Solberg (1938) on the guppy and the medaka (*Oryzias latipes*), respectively, revealed a high degree of radioresistance in the gonads of both sexes. Doses of 1,500 r. to male guppies and 1,980 r. to medakas of both sexes, in the form of x-rays, caused degenerative changes at first, but recovery followed in 1-4 months, coupled with the return of fertility in all.

Observations such as those reviewed indicate that the effect of P^{32} may not be qualitatively the same as that of radiation in other forms, as far as the gonads are concerned. Vivien (1953b, c) advanced the hypothesis that the action of P^{32} on the gonads commences with the diencephalon, where it interferes with neurohypophyseal control, and that it is not reversible owing to neural destruction. A possible objection to this theory might be the radioresistance of nervous tissue (Tullis, 1959).

Summary

1. Total radiothyroidectomy of platyfish, *Xiphophorus maculatus,* 0.5 to 2.0 months old, by means of immersion in solutions of 5.0 mc./200 ml. of I^{131} for 48 hours was successful. although the death rate after such treatment was high.

2. Gonadal development of completely radiothyroidectomized fish was severely retarded and growth in length also was significantly impeded. Vacuolated (=fatty?) liver appeared in both completely and partially thyroidectomized fish and it increased in severity in a progressive manner as time after treatment lengthened. The hematopoietic tissue in the kidneys was reduced, and the blood vessels in the thyroid area showed radiation damage. Pituitary exhaustion appeared to occur.

3. Feeding of these athyroid fish with mammalian thyroid restored them to a normal condition with respect to death rate, growth, sexual development, liver histology and, in part, hematopoietic development. The radiation damage to blood vessels persisted. The time of maturation of males was slightly advanced, and the gonopodia and testes were normal. One female became gravid. These fish were even more normal than their controls in one respect, *viz.*, their pituitaries showed none of the hypertrophy seen in controls with incipient goiter. Elongation of the caudal fin was noted. No exophthalmia occurred.

4. Treatment of these athyroid fish with potassium iodine improved the mortality rate but did not improve growth or reduce the development of vacuolated liver. Gonadal differentiation was slightly less retarded, but no fish approached maturity at the time that the controls reproduced.

5. Fish treated with equivalent, non-thyroidectomizing, irradiation by means of P^{32} were completely normal in growth, secondary sexual development, body shape and visceral histology. Male gonads were completely lacking germ cells, but female gonads were unaffected. The death rate was as high as that shown by I^{131}-treated fish, but appeared to be differently distributed in time from the I^{131} death rate.

6. The injection of adult platyfish with I^{131} led to severe hypothyroidism, but whether it had any effect on the gonads was doubtful. Vacuolated or ceroid-filled livers and enlarged pituitaries were commonly produced.

7. It is concluded that the thyroid plays a significant role in growth, sexual maturation and liver metabolism of the teleost, *Xiphophorus maculatus*. The thyroid also promotes the recovery of hematopoietic tissue after irradiation, and strengthens resistance to shock and disease.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Radiothyroidectomized platyfish, 6 months old, at bottom left, together with normal broodmates—male above and female below, right. Note the hunched shape and drooping fins of the thyroidectomized fish. × ¾. Photo by Sam Dunton, New York Zoological Society.

Fig. 2. Fish harvested from an experiment in which radioiodine-treated fish were treated subsequently with potassium iodide, or were fed mammalian thyroid. All fish were broodmates and 8 were started in each group. **A**, Radioiodine-treated; **B**, Untreated controls; **C**, Radioiodine-treated, fed thyroid; **D**, Radioiodine-treated, KI-treated. Notice the normal size, longer tails and paler coloring of the thyroid-fed fish, in comparison with the untreated controls. Also note the greater survivorship among the KI-treated in comparison with the fish treated solely with radioiodine. × ⅔

Fig. 3. Fish harvested from an experiment in which radioiodine-treated fish subsequently were treated with KI. All fish were broodmates. The initial groups were: **A**, 5 radioiodine-treated; **B**, 4 radioiodine-treated; KI-treated; **C**, 5 untreated controls. The upper fish in group A is shown alive in Fig. 1. Notice the dumpy body shape in all of the radioiodine-treated fish. × 1

Fig. 4. Fish from three experiments in which young platyfish were treated with P^{32}. Untreated controls appear on the left and the corresponding broodmates treated with P^{32} on the right. The P^{32}-treated fish are normal in appearance, and on the average are as large as the controls. × ¾

Plate II

Fig. 1. Stripping film I^{131} radioautograph, showing exposure of emulsion grains over food particles in the pharynx of a platyfish. × 100

Fig. 2. Thyroid follicle in the kidney of a radiothyroidectomized platyfish. This follicle is the only example of renal thyroid tissue found in a radioiodine-treated specimen. × 400

Fig. 3. Regenerated thyroid tissue above the bulbus arteriosus in a radioiodine-treated fish. This female specimen was 7 months old and had been treated with I^{131} when 37 days old. × 100

Fig. 4. Regenerated thyroid tissue above and around the ventral aorta in a radioiodine-treated fish 20 months old. This female had been exposed to I^{131} when 50 days old. In this fish, the regenerated thyroid tissue was almost goitrous in proportion, although the follicles retained their distinct identity from one another. × 100

Fig. 5. Fibrous adhesions developed between the aorta and pericardium in an I^{131}-treated fish. The thyroid area above the pericardium and between the gill chambers is filled with loose stromal tissue. × 100

Fig. 6. Aorta surrounded by thick gelatinous fibrous tissue in a young platyfish treated with radioiodine 24 days prior to fixation. × 200

Plate III

Fig. 1. Kidney of a normal platyfish 10 months old. The dark masses of cells between the tubules are lymphoid tissue; lighter masses consist of nucleated erythrocytes. × 100

Fig. 2. Kidney of a radioiodine-treated broodmate of the normal fish in Fig. 1, at the same age. The kidney tubules are smaller primarily because the fish was much smaller. Chiefly to be noted is the extreme lack of lymphoid cells. × 100

Figs. 3, 4. Examples of "concretions" (arrows) formed within kidney tubules of radioiodine-treated platyfish. Some concretion-like masses not within tubules may represent degenerate tubules. × 100

Fig. 5. Spleen of a normal 6-month-old platyfish. Pigment-laden macrophages are the source of the black spots scattered within the organ. × 100

Fig. 6. Small, deeply basophilic regenerating kidney tubules in an I^{131}-treated platyfish. × 400

Fig. 7. Extremely shrunken spleen (arrow), which lacks all lymphoid elements, of a 6.5-month-old radioiodine-treated fish. The spleen seems more excessively shrunken than it is because the whole animal was subnormal in size. × 100

PLATE IV

FIG. 1. Liver of a normal platyfish. Capillaries are well delineated by the nucleated erythrocytes within them. × 100

FIG. 2. Extremely vacuolated liver and abdominal tissue in a radiothyroidectomized platyfish. The liver is at the right, pancreatic and abdominal connective tissue to the left, and part of the spleen shows at the upper left. × 100

FIG. 3. Ceroid-filled liver in a radiothyroidectomized platyfish. A rounded mass of ceroid-filled macrophages appears next to the blood vessel at the right of center, and the liver cells themselves are packed with ceroid globules. × 100

FIG. 4. Vacuolated liver in a radiothyroidectomized fish, showing part of the gall bladder at the upper left, with gall ducts entering the liver. × 100.

FIG. 5. Pancreas of a normal platyfish. Two loops of intestine are visible at the upper left and lower right. Vacuoles (fat cells?) and large blood vessels appear among the pancreatic masses. × 100

FIG. 6. Pancreas of a radiothyroidectomized platyfish. The pancreatic tissue is compressed by the great amount of abdominal vacuolation. × 100

PLATE V

FIG. 1. Ovarian development in young radioiodine-treated fish. Left: normal ovary of a 4-month-old fish. Right: ovary of an I^{131}-treated broodmate. These sections through the largest diameter of the ovary show the many fewer ova present in the I^{131}-treated fish. × 67

FIG. 2. Testicular development in young radioiodine-treated fish. Left: normal testes of a 4-month-old fish. Many germinal cysts are present. Right: testes of a radioiodine-treated broodmate. No germinal cysts yet have developed. × 286

FIG. 3. Testes of a normal adult male platyfish, 5.5 months old. Many spermatophores are fully formed and grouped in the ducts (bottom right). × 100.

FIG. 4. Testes of a radiothyroidectomized fish, 6 months old. No gonial cysts have developed in this specimen. × 286

FIG. 5. Ovary of a normal female platyfish, 8 months old. Ova at all stages in development may be seen. × 67

FIG. 6. Single ovum found in a radiothyroidectomized fish, 6.5 months old. No yolk deposition has occurred. A portion of intestine lies to the left of this poorly developed ovary. × 100

PLATE VI

FIG. 1. Ovary of a radiothyroidectomized fish, 8 months old. It contained only the three oocytes shown. These ova are in the reticulated stage that normally precedes the appearance of yolk granules. The oviduct appears at the left. × 67

FIG. 2. Testes of a radiothyroidectomized fish, 6.3 months old. In the left lobe of this testis a few germinal cysts had developed and reached early meiotic stages, but the right lobe showed none. × 100

FIG. 3. Nearly mature testis in a radioiodine-treated male fish, 6 months old. This exceptional specimen had no positively identifiable thyroid tissue, and the anal fin was elongated, but totally undifferentiated (see Pl. VII, Fig. 4). × 100

FIG. 4. Mature testis and heavily vacuolated liver in a second radioiodine-treated male fish, 7 months old. This fish had been additionally treated with KI. Its anal fin was totally undifferentiated (See Pl. VII, Fig. 4), and thyroid tissue could not be positively identified. × 100

FIG. 5. Cross-section through the anterior pituitary of a normal platyfish. Compare with Figs. 6 and 7, below, and with Pl. IX, Fig. 7. × 100

FIG. 6. Cross-section through the anterior pituitary of a radioiodine-injected adult platyfish, 15 months old and 6.5 months post-injection. This fish was almost completely thyroidectomized. The large growth of paler basophiles create a striking difference from the normal pituitary, as seen in Fig. 5. × 100

FIG. 7. Cross-section through the anterior pituitary of an untreated adult platyfish, 24-29 months old. The prominent overgrowth of paler basophiles in this case was accompanied by a quiescent and possibly regressive thyroid. The latter may have been the result of the relatively old age of the fish. × 100

PLATE VII

FIG. 1. Gonopodium of a normal adult male platyfish. Compare with Text-fig. 4, and with Figs. 2-4, below. × 25

FIG. 2. Gonopodium of a radiothyroidectomized fish which also was thyroid-fed. Even though the preservation is poorer than that of the fin in Fig. 1, due to fixation of the fish in Bouin's fluid, it may be seen that all of the terminal hooks and spines are present and normally formed. × 25

FIG. 3. Gonopodium of a P^{32}-treated fish. Normal differentiation of all elements is present. × 25

FIG. 4. Elongated but undifferentiated anal fin of a radioiodine-treated male fish, 10 months old. This fish had no demonstrable thyroid and had infantile testes with no germinal cysts (see Fig. 7, below). × 25

FIG. 5. Testicular ducts of a P^{32}-treated male platyfish, 5.5 months old. No germ cells are present, but the duct system is normally complex. The gonopodium of this fish is shown in Fig. 3, above. × 100

FIG. 6. Testes of a 22-day-old platyfish, born to a female injected with radioiodine 19 days before the birth (arrows). This young fish appeared to have a normal thyroid and no other abnormalities. The testes of this fish are almost as large as those of some radiothyroidectomized fish 6 or more months old. Pancreatic tissue appears to the right of the testes, which are centrally located and suspended from the peritoneum. At this age, the two testes are entirely separated; later, in the course of normal development, they become fused. × 100

FIG. 7. Testes of a radiothyroidectomized fish, 10 months old. No gonial cysts were present. These testes are but little larger than those of the 22-day-old male in Fig. 6. The elongated, but undifferentiated, anal fin of this fish appears in Fig. 4, above. × 100

PLATE VIII

FIG. 1. Ovary of a normal pregnant female platyfish. Young oocytes appear at the right, the oviduct at the top, and the neural tube of an early embryo at the arrow. × 100

FIG. 2. Ovary of a radiothyroidectomized, thyroid-fed female. All stages in egg development may be seen in this normally matured ovary. × 100

FIG. 3. Embryo contained in the ovary of a radiothyroidectomized, thyroid-fed platyfish. In this sagittal section, organs such as the eye, brain, kidney, gills and liver may be recognized. × 100

FIG. 4. Section through the pharyngeal area of another embryo contained in the same fish. The arrow points to thyroid follicles. The heart appears at the lower right, and part of the brain at the extreme upper left. × 400

FIG. 5. Ovary of a platyfish treated when young with P^{32}. Oocytes at various stages in development are visible — from the small, darkly basophilic one at the upper left, to the fully yolked egg at the top center. × 100

FIG. 6. Ovary, containing only atretic follicles, of a 17-20 month old platyfish, injected with I^{131} 8.5 months earlier. The oviduct is the darkly stained structure in the central area. ×100.

FIG. 7. Ovary of another 17-20 month old platyfish, injected with I^{131} 8.5 months earlier. In this fish, primary gonial cells appear to be proliferating from the lining of the ovarian cavity, in a manner suggestive of early spermotogenesis (see text). The feminine germinal elements of this ovary appeared to be represented only by atretic follicles. ×100

FIG. 8. Higher power view of the cells proliferating from the ovarian lining in the above specimen. The cells are grouped in balls, as found in the primary gonial cysts of the young testis. ×400

PLATE IX

FIG. 1. Pathological nodule found in the abdominal cavity of a male platyfish, 13 months old, which had been injected with I^{131} 3.5 months earlier. This mass appeared to be attached to the testis, of which a portion may be seen at the upper right. ×100

FIG. 2. Tremendous proliferation of epithelial-like cells in the dorsal pericardium and among the transverse pharyngeal musculature of a platyfish injected with I^{131} 41 days earlier. This female was 10 months old when killed. ×100

FIGS. 3-7. Pathological effects seen in a female platyfish which appeared to represent the most extreme case of hypothyroidism encountered in this study. The fish was 28-29 months old and had been injected with I^{131} 19.5 months before the time of sacrifice.

FIG. 3. Kidney, showing shrunken condition, edematous subcapsular spaces and lack of lymphoid tissue. ×25

FIG. 4. Kidney, under higher power, showing almost complete absence of lymphoid tissue, shrunken tubules and cysticity. Pigment deposits and whorled "nests" are also in evidence. × 100.

FIG. 5. Spleen, very shrunken, owing to reduction of lymphoid elements, and with large pigment deposit in the center. × 100

FIG. 6. Liver, showing vacuolation, fibrous cysts and "nests." The large, rounded mass at the upper left is a large fibrous cyst within the liver. These changes are similar to those of cirrhosis. ×100

FIG. 7. Pituitary, with an overgrowth of paler basophilic elements of such magnitude that it might well be classed as a tumor. Compare with the normal pituitary shown in Pl. VI, Fig. 5, which is a section taken at the same level. ×100